Національний університет біоресурсів і природокористування України

БІОЛОГІЧНІ СИСТЕМИ:
Теорія та Інновації

Том 17, № 1
2026

Київ 2026

ISSN 2706-8382
e-ISSN 2706-8390

Засновник:
Національний університет біоресурсів і природокористування України

Рік заснування: 2010

Рекомендовано до друку та поширення
через мережу Інтернет Вченою радою
Національного університету біоресурсів і природокористування України
(протокол № 9 від 26 лютого 2026 р.)

Державна реєстрація: Ідентифікатор медіа R30-02292.
Рішення Національної Ради України
з питань телебачення і радіомовлення
№ 1795, протокол № 31 від 21.12.2023 р.

Журнал включено до категорії «Б» Переліку наукових фахових видань України,
відповідно до якого можуть публікуватися результати дисертаційних робіт на здобуття наукових
ступенів доктора та кандидата біологічних наук зі спеціальностей
(наказ Міністерства освіти і науки України № 409 від 6 березня 2020 року):
091 – Біологія; 101 – Екологія; 162 – Біотехнології та біоінженерія; 202 – Захист і карантин рослин

Кластер журналу, відповідно до Наказу Міністерства освіти і науки України 19 лютого 2026 року:
Біологія, біотехнології, медицина та реабілітація.

Журнал представлено у міжнародних наукометричних базах даних,
репозитаріях та пошукових системах:
Open Ukrainian Citation Index, University of Hull Library, University of Oslo Library, WorldCat,
Crossref, Національна бібліотека України імені В.І. Вернадського, BASE, EBSCO,
Google Академія, Ulrichsweb Global Serials Directory, UCSB Library, Dimensions, Litmaps, AGRIS,
CABI

Адреса редакції:
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https://bioscience.com.ua/uk

National University of Life and Environmental Sciences of Ukraine

BIOLOGICAL SYSTEMS: Theory and Innovation

Vol. 17, No. 1
2026

Kyiv 2026

ISSN 2706-8382
e-ISSN 2706-8390

Founder:
National University of Life and Environmental Sciences of Ukraine

Year of foundation: 2010

Recommended for printing and distribution
via the Internet by the Academic Council
of National University of Life and Environmental Sciences of Ukraine
(Minutes No. 9 of February 26, 2026)

State registration: Media identifier R30-02292.
Decision of the National Council of Television
and Radio Broadcasting of Ukraine
No. 1795, Minutes No. 31, dated 21.12.2023.

The journal is included in category “B” of the List of scientific professional publications of Ukraine, in which the results of dissertations for the degree of doctor and candidate of biological sciences can be published in the following specialties
(Order of the Ministry of Education and Science of Ukraine No. 409 dated 06.03.2020):
0511 – Biology; 0521 – Environmental sciences; 0588 – Interdisciplinary programmes and qualifications involving natural sciences, mathematics and statistics; 0712 – Environmental protection technology

Journal cluster, according to the Order of the Ministry of Education and Science
of Ukraine dated February 19, 2026: Biology, Biotechnologies, Medicine and Rehabilitation.

The journal is presented in the international scientometric databases, repositories and scientific systems:
Open Ukrainian Citation Index, University of Hull Library, University of Oslo Library, WorldCat, Crossref, Vernadsky National Library of Ukraine, BASE, EBSCO, Google Scholar, Ulrichsweb Global Serials Directory, UCSB Library, Dimensions, Litmaps, AGRIS, CABI

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Зміст/Contents

Received: 07.10.2025 Revised: 21.01.2026 Accepted: 26.02.2026 Published: 07.04.2026

BIOLOGICAL SYSTEMS: Theory and Innovation

ISSN 2706-8382
e-ISSN 2706-8390

Journal homepage: https://bioscience.com.ua/en
Vol. 17, No. 1, 2026, 10-24

UDC 574.91:581.9:502/504.064.3:528.9(477.86–751.2/.3)

DOI: 10.31548/biologiya/1.2026.10

Development of an electronic questionnaire for field studies of invasive plant species in the Carpathian National Nature Park

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Abstract. The relevance of the study was conditioned by the need for standardised, reproducible, and geospatially linked monitoring of invasive alien plant species in the complex mountain landscapes of the Carpathian National Nature Park, where the combination of high-altitude zoning, diversity of habitats, and natural and anthropogenic distribution corridors creates increased risks of their naturalisation and expansion. The purpose of the study was to create a standardised questionnaire to unify the process of recording data on invasive species, ensure correct geospatial binding of observations, and establish prerequisites for their further spatial analysis in the ArcGIS environment. The survey form, developed in ArcGIS Survey123, covered a wide range of ecological, biological, and abiotic parameters, including data on the taxonomic affiliation of the species, projective coverage, ontogenetic state of the population, soil characteristics, microclimatic indicators, and the level of anthropogenic impact. The proposed approach allowed improving the accuracy of monitoring invasive species and integrating the data obtained into national observation databases. The design of the questionnaire was based on the principles of maximum compatibility, followed by analysis in a geographic information system and publication in global biodiversity repositories. This approach opened up the possibility of comparative analysis, merging with citizen observations, and using data to assess risks and prioritise invasion responses. Methodically, the questionnaire covered 8 logical sections: (1) general information and geo-linking; (2) description of the site with classes of ecosystem types in accordance with contemporary global typology; (3) characteristics of the population of an invasive species, including area, density, age structure, dynamics; (4) ontogenetic spectrum with quantitative accounting by stage; (5) soil and microclimatic indicators with the possibility of field determination of acidity and humidity; (6) threats and damage to plants and ecosystems; (7) soil litter as a potential seed bank; (8) additional comments

Suggested Citation:

Nasadiuk, P., Mamchur, Z., & Tymchuk, O. (2026). Development of an electronic questionnaire for field studies of invasive plant species in the Carpathian National Nature Park. *Biological Systems: Theory and Innovation*, 17(1), 10-24. doi: 10.31548/biologiya/1.2026.10.

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and photo documentation. It provided for collecting photos from different angles, uploading them with spatial reference to records, and forming a complete digital footprint for further quality audit

Keywords: phytoinvasions; monitoring; alien species biota; habitats; biodiversity; ecosystems; nature reserve fund

INTRODUCTION

Invasive alien plant species (IAPS) are one of the key factors for biodiversity loss and ecosystem disruption, which necessitates their early detection, systematic monitoring, and coordinated management, particularly at the level of protected areas (IUCN, 2020). Invasive species can significantly transform the structure of natural communities, displace native flora, disrupt biogeochemical cycles and trophic relationships, and reduce the ecological stability of biocoenoses. As noted by P.P. Nasadiuk & Z.I. Mamchur (2025), in the European policy and management context, the invasion response framework is supported by taxon lists and prevention, early detection, and management procedures, further highlighting the importance of standardised primary data. For mountain ecosystems of the Carpathians, the urgency of the problem is compounded by the mosaic of high-altitude belts, the variety of habitat types, and recreational load, which forms multidirectional vectors of IAPS propagation along linear corridors (roads, trails, river valleys), as emphasised by L.A. Santoianni *et al*. (2024). The study by M. Renčo *et al*. (2024) showed that roadsides and hiking trails act as important linear corridors for the distribution of atypical, in particular, invasive flora elements in natural ecosystems. Violation of the soil cover, changes in the microclimate, increased illumination and regular anthropogenic impact create favourable conditions for the penetration, consolidation, and further spread of alien species, which can lead to transformation of the species composition of plant communities and changes in the structure of local biocoenoses.

K. Najberek *et al*. (2024) noted that mountain roads act as key corridors for the spread of invasive plants, as soil disturbances and other anthropogenic factors near roads significantly increase the likelihood of alien species appearing. It was found that their distribution is most often associated with warm, well-lit and disturbed ecosystems, while natural communities far from roads have higher resistance to invasion. Analysis by P.K. Rai & J.S. Singh (2020) focuses on the environmental, socio-economic, and medical implications of the spread of invasive alien plant species. The researchers found that such species significantly alter the structure of ecosystems, reduce biodiversity, disrupt ecosystem services, and can pose risks to human health, highlighting the need for integrated approaches to their monitoring and management. For the Carpathian region, one of the illustrative examples is the influence of *Impatiens parviflora* in beech forests. For the territory of the Carpathian National Nature Park (CNNP), the issue of detection and control of invasive species is of particular importance, given its importance for the preservation of natural ecosystems, the protection of rare, relict, and endemic plant species, and for the tourist attractiveness of the territory and the high number of visitors throughout the year (Danko *et al*., 2024). Despite the high scientific value of this territory, information on the distribution of IAPS within the CNNP remains limited and fragmented.

As emphasised by E.M. Geurts *et al*. (2023) and P.P. Nasadiuk & Z.I. Mamchur (2025), the available open observation platforms (in particular GBIF, iNaturalist, UkrBIN) provide an important array of data, but in mountainous areas there are often spatial gaps and heterogeneity of collection protocols, which makes it difficult to compare and use records in application management. This, in turn, creates the basis for population analysis of time series construction and dynamic modelling (subject to long-term surveys), including assessment of ecosystem characteristics, quantitative assessment of the effects of invasive transformer species and indication of changes in the provision and maintenance of ecosystem services. In turn, R. Montagnetti & G. Guarino (2021) described the benefits of using the QField mobile application for field geo-linked data collection, in particular, that it allows transferring projects from desktop GIS (QGIS) to Android devices, significantly reducing the time and cost of data entry, eliminating the need for paper documentation, and providing efficient digital registration and analysis of spatial information directly in the field. As noted by M. Silva del Pozo *et al*. (2023), the development of a digital questionnaire that combines standardised field protocols with a geographic information system (GIS) and a GIS-oriented data structure and the ability to publish in open repositories is a prerequisite for effective monitoring and adaptive management of bioinvasions. The purpose of the study was to develop and substantiate a GIS-oriented digital questionnaire for monitoring invasive plants in CNNP with the integration of geoanalysis and the preparation of data for open exchange.

MATERIALS AND METHODS

The questionnaire "Populations – field study of invasive plants on the territory of CNNP" was designed specifically for the conditions of the CNNP, taking into consideration orography, slope exposures, altitude zones, and a set of habitats characteristic of the park territory. The terminology of relief elements (ridge, slope, foothill, hollow, scree, terrace, shoreline), and soils and habitats was established based on generalised data on abiotic conditions and nature chronicles of the park, in particular, R.L. Kravchynskyi *et al*. (2025) and Carpathian National Nature Park (n.d.). The standardised design of the questionnaire combined population indicators (density, generativity, age structure, if necessary – vital elements) with a phytocoenotic context (Braun-Blanquet tiering and coverage) and related abiotic characteristics (soil, microclimate, habitat type). The development of the questionnaire was based on methodological recommendations for field research developed by the Ivan Franko Lviv National University and supplemented with author's thematic blocks. The questionnaire was implemented in the ArcGIS Survey123 environment, which is one of the ArcGIS Pro products as a mobile form with the ability to collect and then synchronise data offline. The design of the field structure, their binding nature, and response types was carried out in accordance with the recommendations of the platform for creating questionnaires and managing geo-linking/media data – ArcGIS Survey123 (n.d.). The choice of Survey123 was conditioned by full compatibility with GISenvironment: export of data to tabular formats (CSV/Excel), file geobases, and Feature Layer, followed by analysis and mapping in ArcGIS Pro (with the connection of background layers, digital terrain models, cadastral and thematic layers). The form contained 9 sections:

1. General data: executor, date/time (automatically).

2. Site description: Site description included geomorphological and microrelief features, exposure, steepness, altitude, and classification of the ecosystem type. For the typification of ecosystems, consistent classes were used, correlated with the functional typology of Earth ecosystems in accordance with D.A. Keith *et al*. (2022). The size of the accounting (model) area was set by a discrete set of standard sizes, which was consistent with approaches to choosing the size of test areas in European phytosociological practice, in particular by M. Chytrý & Z. Otýpková (2003). The spatial boundaries of the "study area" were fixed by a polygon or point on the map, which corresponded to detailed mapping approaches for studying the structure of populations and the spatial grouping organisation by M.B. Gaponenko & A.M. Gnatiuk (2013).

3. Population characteristics: taxon (Lat./Eng.), the degree of invasiveness, including the "transformer" category (according to V.V. Protopopova & M.V. Shevera (2019)), habitat area, density within the model plot, age structure, presence of generative individuals, assessment of dynamics during repeated visits. The methodological basis for the establishment of these blocks was made up of recommendations on population ecology and methodology for studying plant populations by O.S. Abduloieva & N.I. Karpenko (2012), L. Zavialova (2019), V. Kyyak & A. Malynovsky (2020).

4. Ontogenetic spectrum: quantitative accounting by stage *s*, *p*, *j*, *im*, *v*, *g1-g3*, *ss*, *s*, allowed assessing the progression of invasion, interpreting the life state of the population, its recovery, and potential expansion trajectory. If necessary, it is possible to use an algorithm for assessing the vitality of individuals and the vitality structure of populations. The combination of the ontogenetic spectrum with vital approaches provides a transition from a static "section" to a dynamic assessment of population processes.

5. Coverage and phytocoenotic context assessment: the Braun-Blanquet coverage scale with separate sub-blocks for tree, shrub, grass, moss-lichen tiers and the proportion of areas without vegetation was used to assess the participation of

invasive species and vertical vegetation structure. The field application of the scale was consistent with the standard Braun-Blanquet operating procedure and contemporary approaches to its interpretation by I.V. Prystupa (2017), Realising dynamic value… (2021). Accounting for coverage by Braun-Blanquet for different tiers of vegetation allowed transferring the results to the plane of phytosociological descriptions and comparing them between locations and seasons.

6. Soil and microclimatic parameters: soil type, field humidity (touch test/instrument), pH, soil and air temperature, illumination, fertility indicators, sampling methods and measurement accuracy requirements were consistent with the guidelines for harmonised soil monitoring and field description by N. Cools & B. De Vos (2020).

7. Threats and damage: erosion, flooding, fires, windbreaks, grazing, recreational load, forestry activities, etc. – in the logic of the link "disturbance factors → habitat vulnerability", considering the role of linear corridors and attendance in the spread of IAPS. The list of threats was checked against local sources of CNNP and for zoning vulnerabilities in habitats, including R.L. Kravchynskyi *et al*. (2025) and Carpathian National Nature Park (n.d.). The IAPS risk assessment was provided in accordance with the guidelines of Order of the Ministry of Environmental Protection and Natural Resources of Ukraine No. 290 (2024) and Environmental Impact Classification for Alien Taxa (EICAT) IUCN (2020).

8. Soil litter: thickness, composition, degree of decomposition, humidity, presence of seedlings/seeds of IAPS. The block was critical for transformer species that modify phytocoenoses through seed banks and changes in fuel load; methodological guidelines – International Co-Operational Programme on Assessment and Monitoring of Air Pollution Effects on Forests (ICP Forests, n.d.) and field guidelines for soil description, and classification of high activity of invasive species, in particular by H.E. Roy *et al*. (2024).

9. Additional comments/media to clarify the context of the post.

The spatial boundaries of the study area were fixed by a polygon on the map; model areas were defined by discrete dimensions (0.5 × 0.5; 1 × 1; 2 × 2; 5 × 5; 10 × 10 m), which corresponds to the practice of European phytosociological studies by J. DánielFerreira *et al*. (2024) and M. Chytrý & Z. Otýpková (2003). For linear sections (roadsides/trails, river valleys), the route bypass was combined with detailed cell mapping, according to the method of spatial analysis of populations by M.B. Gaponenko & A.M. Gnatiuk (2013). The inclusion of corridor habitats was supported by empirical data on the role of infrastructure in the spread of IAPS provided by M. Jurišić *et al.* (2023) and J. DánielFerreira *et al.* (2024). There were: mandatory fields for key attributes; questions with standardised answer options; photo recording from different angles; automatic recording of coordinates/altitude/time; export to tables and GISlayers for further analysis in ArcGIS Pro (including the connection of DEM, soil, hydrographic network, and other layers).

The developed electronic questionnaire was tested in June-August 2025 on the territory of the CNNP. Testing was conducted along designated hiking trails within the regulated recreation zone, specifically at the following locations: Divochi Slozy, Animal enclosure farm, Dovbush Trail, Polonyna Borsuchyna, Mount Yahidna, Mount Makovytsia, Polonyna Chornohorytsia, the Rudiak raised bog, and partially along the routes to Mount Hoverla and Mount Shpytsi Additionally, the study covered areas of stationary recreation, including the recreational sites Zhonka, Berizhok, Bohonchyk, Tyrlych, Zarosliak, Lisnychivka, Hoverla, Dzherelo, Zhenets, Vorotyshche, and the trout farm of the Hoverlianske Forestry and other adjacent territories. Field studies were conducted in a wide altitude range and covered various types of mountain ecosystems, in particular beech and fir-beech forests, spruce forests of the upper mountain belt, swamp complexes and coastal-aquatic biotopes, and anthropogenically transformed habitats (roadsides, forest edges, parking/recreation areas). Such an ecosystem spectrum allowed testing the functionality of the questionnaire under conditions of different vegetation cover structure, mosaic of habitats, and variability of microclimatic parameters. Testing was carried out both in areas with stable cellular coverage, and in areas with its complete or partial absence, which allowed checking the correctness of offline data collection, their further synchronisation, and the preservation of geospatial binding of records. Testing confirmed the operability of the form in various field conditions, including hard-to-reach mountain areas and areas of intense recreational load.

RESULTS AND DISCUSSION

The developed electronic questionnaire provides structured recording of both individual individuals and full-fledged populations/foci of invasive plant species using point and polygonal geometry. Table 1 shows the structure of the questionnaire, where each parameter is accompanied by a brief instruction for field determination, and attached photo recording samples. Due to the implementation in ArcGIS Survey123, all records are exported to Tabular formats (CSV/Excel) and directly exported to GISenvironment as Feature Layer layers for further mapping, spatial analysis, and attaching external layers in ArcGIS Pro (terrain models, soil models, hydrographic network models, etc.).

Table 1. Populations – field study of invasive plants on the territory of CNNP

Parameter/ Question	Hint	Answer/Options to choose from
General data		
Researcher name		Text
Date and time		Automatically
Description of the study area		
Location name	Specify the name of the area, route, or specific location	
Microrelief	• Ridge – the highest line of a ridge or hill. • Slope – sloping part of a mountain or hill. • Foothill – lower part of the slope. • Terrace – flat ledge on a slope or above a river. • Hollow – depression in the terrain, often wet. • Shoreline – areas adjacent to the riverbed. • Rocks – open outcrops of hard rock. • Scree – embankment of stone fragments under a slope.	Ridge/Slope/Foothill/Terrace/Hollow/ Shoreline/Rocks/Scree
Where exactly is the study area located?	Roadsides, trails, forests, meadows (up to 2 m from the edge), forest, meadow, shoreline, etc.	Text
Altitude above sea level	If information is available, or it can be added later during GIS analysis	Number
Slope steepness	Methods: Clinometer (or built-in compass) – accurate measurement of the tilt angle. Mobile applications (for example, Clinometer, Theodolite, or GPS applications with slope detection function). 0-3° – plain 3-7° – gentle, barely noticeable slope 7-15° – noticeable, but easy to walk 15-25° – steep, requires effort 25-35° – very steep, sometimes requires support >35° – steep slope/cliff, almost vertical	0-3° 3-7° 7-15° 15-25° 25-35° >35°
Slope exposure	What is required: a compass or an application that detects slope exposure on the phone. How to determine: 1. Stand face down the slope (in the direction where the water flows). 2. Measure the azimuth (angle in degrees from north). 3. Translate to direction: • 0° = N (north) • 45° = NE (northeast) • 90° = E (east) • 135° = SE (southeast) • 180° = S (south) • 225° = SW (southwest) • 270° = W (west) • 315° = NW (northwest) If the slope is almost flat → "plain".	N NE E SE S SW W NW Plain

Table 1. Continued

Parameter/ Question	Hint	Answer/Options to choose from
Ecosystem type	Select	Alpine meadows/Subalpine meadows/ Scattered woodland/Coniferous forests/ Beech forests/Mixed forests/Wetlands/Rocky areas/Forest edges/Mountain pastures/ Water bodies/Urbanised areas/ Agricultural landscape/Other
Location description	Specify important additional information	Text
Photo shared	General photo of the population at the growth site	Add a photo
Boundaries of the study area	Draw a polygon on the map that marks the boundaries of the entire study area (NOT an accounting, model area)	Draw a polygon on the map
Size of the accounting (model) area (m)	Select	0.5 × 0.5 1 × 1 2 × 2 5 × 5 10 × 10
Population characteristics (single plant)		
Taxon	Latin name of the invasive species (dominant invasive species on the site)	Text
Household name	Ukrainian household name	Text
Degree of invasiveness	Select	Transformer Highly invasive Invasive Introduced Naturalised Undetermined
Population occupied area (m^2)	Approximate area of the entire plot within which accounting (model) squares are laid (m^2)	Number
Stage of ontogenesis	Question only for fixing a single plant	s – seed; p – seedling; j – juvenile; im – immature; v – vegetative adult; g1 – generative 1 (beginning of flowering, single flowers); g2 – generative 2 (mass flowering); g3 – generative 3 (fruiting, seeds); ss – subsenile plant (ageing, losing generative characteristics); s – senile plant (end of life cycle)
Age structure	Mostly young/Mixed/Mostly old	Text
Presence of generative individuals	Select	Yes No Other
Population dynamics assessment	This question applies only to repeated surveys of the same site conducted in different periods. If this is the first survey, the field remains empty or "Not applicable" is selected	Growing Stable Decreases Unknown Not applicable
Population density	Individuals per m^2 – for an accounting (model) plot	Number
Braun-Blanquet coverage for the species under study	Select	i – only one individual r – single individuals, coverage <1% + – multiple individuals, coverage <1% 1 – numerous individuals, coverage 1-5% 2 – coverage 6-25% 3 – coverage 26-50% 4 – coverage 51-75% 5 – coverage 76-100%
T – Braun-Blanquet coverage for wood tier	Tent (upper wooden tier). Tall trees that form the forest crown (spruce, beech, fir)	0% <1% 1-5% 6-25% 26-50% 51-75% 76-100%

Table 1. Continued

Parameter/ Question	Hint	Answer/Options to choose from
S – Braun-Blanquet coverage for shrub tier	Undergrowth Bushes, young trees 0.5-5 m May also include undergrowth of tree species	0% <1% 1-5% 6-25% 26-50% 51-75% 76-100%
H – Braun-Blanquet coverage for grass tier	All grass species, including young seedlings <0.5 m Here, the coverage of invasive species is most often determined	0% <1% 1-5% 6-25% 26-50% 51-75% 76-100%
B – Braun-Blanquet coverage for moss-lichen tier	All herbaceous species, including young seedlings <0.5 m	0% <1% 1-5% 6-25% 26-50% 51-75% 76-100%
W – Braun-Blanquet coverage for areas without vegetation		0% < 1% 1-5% 6-25% 26-50% 51-75% 76-100%
Photo square of the accounting (model) plot		Add a photo
Other invasive plant species	Specify other invasive plant species in the study area	Text
Other plant species	Specify other plant species in the study area	Text
Presence of other groups of organisms	Mark all available groups, additional information can be provided in the following question (multiple choice)	Lichens Fungi Insects Arachnids Molluscs Amphibians Reptiles Mammals Other
Description of other groups of organisms (if additional information is available)	Features and names, if any, and other groups of organisms that are not specified in the previous question	Text
Additional photo		Add a photo
Ontogenetic spectrum of the accounting (model) plot		
s – seed	Quantity per m^2	Number
p – seedling	Number of individuals per m^2	Number
j – juvenile	Number of individuals per m^2	Number
im – immature	Number of individuals per m^2	Number
v – vegetative adult	Number of individuals per m^2	Number
g1 – generative 1 (beginning of flowering, single flowers)	Number of individuals per m^2	Number
g2 – generative 2 (mass flowering)	Number of individuals per m^2	Number

Table 1. Continued

Parameter/ Question	Hint	Answer/Options to choose from
g3 – generative 3 (fruiting, seeds)	Number of individuals per m^2	Number
ss – subsenile plant (ageing, losing generative characteristics)	Number of individuals per m^2	Number
s – senile plant (end of life cycle)	Number of individuals per m^2	Number
Photo square of the accounting (model) plot		Add a photo
Photo flower	Photo of a plant flower (if any) or fruit (seed)	
Photo sheet	Close-up photo of the sheet	Add a photo
Additional photo	Additional photo that contains important information	Add a photo
Comment on the population structure	Additional information and features	
Soil characteristics		
Soil type	Specify according to the prompts below, including additional information if necessary: • Mountain-forest brown soils: forest zones (beech, fir, spruce); dark brown in colour, loose in texture, high in humus, with stony inclusions. • Soddy-podzolic: lower parts of slopes, forest edges; a lighter upper horizon, beneath which lies a distinct whitish podzolic layer. • Mountain-meadow: subalpine and alpine meadows; dark humus horizon, well-developed turf, high moisture content. • Peat (swamp): swamps, sphagnum areas; dark brown or black colour, fibrous structure, saturated with water, elastic. • Gravelly/stony: scree, steep slopes; a small amount of fine soil, rock fragments predominate. • Rendzinas (on limestone): limestone outcrops and scree; a thin dark humus horizon over limestone fragments; positive reaction with HCl (effervescence). • Loams: slopes, terraces; sticky when wet, retain shape when compressed. • Sandy loams: scree, deluvial deposits; easily crumbling, do not retain their shape, the sand fraction is well felt.	Text
Soil moisture	Field touch test	Dry Moderate Wet Waterlogged Water on the surface
Soil pH	According to the device's indicators (if any)	Number
Soil moisture	According to the device's indicators (if any)	Number
Soil temperature	According to the device's indicators (if any)	Number
Soil illumination	According to the device's indicators (if any)	Number
Soil fertility indicator	According to the device's indicators (if any)	Number
Air temperature	According to the device's indicators (if any)	Number
Air humidity	According to the device's indicators (if any)	Number
Threats or damage to the population / ecosystem		
Signs of drought	The studied and surrounding plants are withered, wilted, the soil is hard and dry, etc.	Yes/No
Signs of flooding	The soil is waterlogged, water on the surface, other signs of flooding of the site (presence of eroded soil, deposited sand, etc.)	Yes/No

Table 1. Continued

Parameter/ Question	Hint	Answer/Options to choose from
Signs of fire	Burned area, or the presence of burned bushes, trees, etc. – except for specially designated places for bonfires	Yes/No
Signs of soil erosion		Missing/Local/Medium/Strong
Signs of landslides		Yes/No
Signs of windfall		Yes/No
Signs of grazing	Traces of livestock (driving cattle), animal droppings, grazed areas	Yes/No
Signs of recreational stress	Multiple choice	Garbage/Trails/Camping/Damage/Campfires/ Transportation/Missing/Other
Signs of forestry/ deforestation	Multiple choice	Fresh stumps/Old stumps/Track/ Skidding tracks/Wood warehouses/ Cutting area/Sanitary logging/Other
Additional information	If necessary	
Photo	If necessary	
Plant damage		
Type of damage	Multiple choice	Pests/Fungi/Gnawing/Icing/Drought/ None/Other
Detailing of damage	Include both natural (pests, icing) and recreational (cutting, trampling)	
Photo of a damaged plant		
Soil litter		
Thickness of soil litter		Less than 1 cm/1-3 cm/3-5 cm/>5 cm
Litter composition		Leaves/Needles/Grass residues/Mosses/ Lichens/Branches/Bark/Debris/Other
Degree of decomposition		Fresh/Partially decomposed /Fully decomposed
Litter humidity		Dry/Moderately wet/Wet
Presence of seedlings/seeds of invasive species in the litter		Yes/No
Photo		
Additional comments		
Additional comments		Text

Note: GPS – Global Positioning System
Source: compiled by the authors

Table 1 contains blocks of questions about population structure, Braun-Blanquet vegetation cover, ontogenetic composition, related species, soil characteristics, and anthropogenic or natural impact factors. This structure provides a systematic collection of quantitative and qualitative data necessary for further analysis of the distribution and state of invasive plant populations. The questionnaire is suitable for use in citizen science programmes, considering the EU policy and organisational framework, in particular, Commission Implementing Regulation (EU) 2024/574 (2024) and V. Lush *et al*. (2024). To this end, the system features multiple-choice questions, simple hints, mandatory photos, and geotagging of entries. A total of 44 localities of invasive plant populations and 36 localities of single growth of the following invasive plants were documented: *Impatiens glandulifera*, *I. parviflora*, *Heracleum sosnowskyi*, *Bidens frondosa*, *Echinocystis lobata*, *Reynoutria japonica*, *Erigeron annuus*, *E. canadensis*, *Robinia pseudoacacia*, *Solidago canadensis*, *Helianthus tuberosus*, *Rudbeckia laciniata*. Figure 1 shows the ArcGIS Survey123 interface (Data tab) with a map and attribute table of completed entries in the invasive plant questionnaire in CNNP.

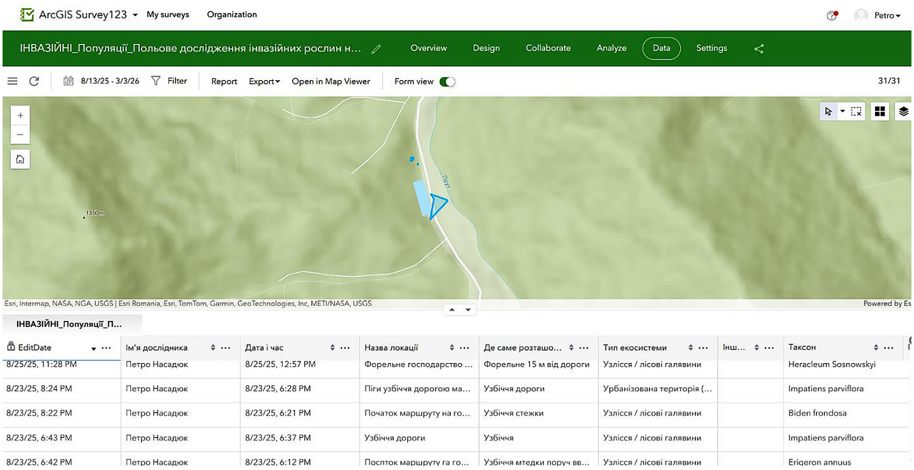

Figure 1. Example of a report from ArcGIS Survey123
Source: compiled by the authors

Figure 1 shows several geo-linked observations (blue polygons) along the road infrastructure and riverbed; the table showed key fields: date and time of the study, name of the researcher, name of the location, type of ecosystem, and the taxon. Among the recorded taxa – *Heracleum Sosnovskyi*, *Impatiens parviflora*, *Bidens frondosa*, *Erigeron annuus*, fixation sites were specified as roadsides/trails, forest edges, etc., which indicated the dominance of linear and marginal habitats in the sample. Records were grouped by routes (for example, "Trout farm") and had timestamps (25.08.2025), which confirmed field data entry with subsequent viewing and analysis on the map. The proposed questionnaire can be easily adapted for recording and population analysis of habitats of rare, relict, and endemic species both on the territory of the CNNP and in other protected areas of Ukrainian Carpathians. To do this, in the section "Population characteristics", it was proposed to remove the parameter "Degree of invasiveness" and supplement the form with a specialised block of questions focused on the protected status and demographic indicators of such species (Table 2).

Table 2. Block of questions for rare, relict, and endemic species

Is the species endemic to the Carpathians?	Select	Yes/No/Unknown
Is the species relict for the Carpathians?	Select	Yes/No/Unknown
Is the species listed in the Red Book of Ukraine?	Select	Yes/No/Unknown/Needs verification
Does the species have an international protected status?	Multiple choice	IUCN (Specify the category in the following question: CR, EN, VU, NT, LC)/CITES/Bern Convention/Emerald Network/None/Other
Additional information on international protection status	Additional information (also specify the IUCN category: CR, EN, VU, NT, LC – if applicable)	Text

Note: IUCN – International Union for Conservation of Nature; CR – Critically Endangered; EN – Endangered; VU – Vulnerable; NT – Near Threatened; LC – Least Concern; CITES – Convention on International Trade in Endangered Species of Wild Fauna and Flora
Source: compiled by the authors

Table 2 systematises the collection of data on rare, relict, and endemic species, including national and international protected status and the possibility of additional detail. In the context of interpreting population data for IAPS, the study was based on the methodological foundations summarised by V. Kyyak & A. Malynovsky (2020), who considered the population as a basic unit of accounting for biodiversity and insist on assessing the structure (age/ontogenetic, spatial, sexual), self-renewal and strategies of the species in specific ecological niches; they considered vitality to be an integral indicator of the state, synthesising key individual and group parameters of growth, development, and reproduction. This established the basis of the survey form: blocks on the ontogenetic spectrum, the proportion of generative individuals, density, and coverage (as a proxy for vital activity), and on the regeneration niche (soil litter/seed bank, microsites), allow tracking intrapopulation diversity and self-maintenance mechanisms in different CNNP habitats. In particular, the typical stages of degradation of rare populations described by the researchers under pessimistic conditions (simplification of spatial structure, ageing or pseudo-rejuvenation, drop in vitality, extinction of generation, decrease in area/number/density) were operationalised through mandatory mapping of population polygons, fixing the dynamics of the generative contour, which is consistent with the authors' approaches to diagnosing population trends in mountain systems. Such integration of population and environmental indicators in the digital questionnaire allows continuing the line of standardised field collection and moving from one-time statements to monitoring the vitality/self-healing of IAPS populations in space and time. P. Pyšek *et al*. (2020) summarised that biological invasions pose a large-scale systemic risk and require early detection and coordinated action, with standardised primary data being key for policy and management. The proposed tool is consistent with these results, since it provides the most standardised, geospatial data collection and the ability to quickly map invasion foci. Simultaneously, the difference lies in the focus: within the framework of this study, the emphasis was placed on operational implementation (form structure, reference books, export to GIS), while P. Pyšek *et al*. established mainly conceptuallypolitical framework. K. Ingenloff (2025) detailed approaches to updating and publishing monitoring data in Darwin Core using the Humboldt Extension, suitable for event recording and protocol documentation.

The proposed field and metadata mapping complies with these practices and ensures that the collected records are ready for integration into GBIF/UkrBIN. M. Jurišić *et al*. (2023) showed that zoning of environmental vulnerability combined with traffic forecasting allows identifying "hot spots" of risk in protected areas, which was especially important for mountain parks. In this study, this was considered through mandatory localisation fields (roadsides, hiking trails, river valleys) and site polygon mapping in Survey123. A common feature was the spatially oriented approach to risk assessment; the difference lied in the subject of observation: here, the focus was on IAPS populations, whereas M. Jurišić *et al.* analysed the vulnerability of territories in general. J. Dániel-Ferreira *et al*. (2024) demonstrated by field studies in central Sweden that roadsides with high environmental value are vulnerable to expansion of *Lupinus polyphyllus*, and linear media act as effective channels for its propagation. The proposed questionnaire institutionalised this sensitivity through the field "Where exactly is the site located?" and a threat assessment unit that allows easily aggregating records for further corridor analysis. The difference is in the breadth of taxa: the study by Swedish researchers focused on one species, while the proposed form covered the entire spectrum of IAPS, including transformer species. A. Mihaly & V. Roman (2024) showed that spatial kits for monitoring invasive tree species in the forests of Zakarpattia allow localising foci and supporting management decisions. The developed model confirmed the validity of spatially oriented data, but extended it to non-seasonal herbaceous and shrubby invasions by combining indicators of cover, ontogenetic structure, and soil characteristics. Realising dynamic value... (2021) formalised the standard operating procedure (SOP 26) to apply the Braun-Blanquet scale in field protocols, emphasising the unification of procedures and the possibility of comparable assessments between different projects. In this paper, the scale was integrated as the core of the phytocoenotic block with separate accounting of vegetation tiers, which fully corresponds to SOP 26 and simplifies further transformation of ordinal classes into quantitative indicators for analytical needs.

CONCLUSIONS

The developed electronic questionnaire in the ArcGIS Survey123 environment provides standardised, reproducible, and geospatially linked collection of primary data on IAPS on the territory of CNNP. The structure of the tool integrates population indicators (density, ontogenetic spectrum), phytocoenotic context (Braun-Blanquet coverage assessment divided into tiers), abiotic characteristics (soil and microclimatic parameters, soil litter status), and the threat and damage module. This approach increased the explanatory capacity of field observations of the stages of the invasive process and formed a reliable basis for adaptive management. The questionnaire was adapted to the specifics of CNNP mountain landscapes: orography, slope exposures, altitude zones, and the most common ecosystem types and disturbance factors for the park were considered. Mandatory fields, controlled reference books, photo audits, and automatic geo-linking were implemented, which reduced inter-observer variability and facilitates quality control. The native integration with ArcGIS Pro allows quickly mapping points and polygons, and connecting external GIS layers (DEN, soil, hydrographic network, infrastructure), and organisation of further spatial analysis. The study demonstrated the effectiveness of the transition from episodic observations to systematic monitoring of invasive species, which allowed accurate detection and mapping of foci, monitoring population dynamics during repeated surveys, comparing indicators between sites and seasons, and quickly generating data for management decisions and reporting. Further development should be directed to the introduction of stratified sampling by altitude zones and habitat types, regular repeated surveys, expansion of sensors (GNSS, pH, humidity, PAR), and setting up an automated data publishing pipeline in Darwin Core. This evolution will strengthen evidence-based solutions for the prevention, early detection, and control of invasive species in the Carpathian mountain ecosystems. The tool can also be used for early detection of new invasive taxa, mapping of overgrowth foci, monitoring the effectiveness of spread control measures, assessing impacts on rare and sensitive environments, and for operational reporting and management planning with cost minimisation and unification of data circulation.

ACKNOWLEDGEMENTS

None.

FUNDING

None.

CONFLICT OF INTEREST

None.

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Розробка електронного опитувальника для польових досліджень інвазійних видів рослин на території Карпатського національного природного парку

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Анотація. Актуальність дослідження зумовлена потребою у стандартизованому, відтворюваному та геопросторово прив'язаному моніторингу інвазійних чужорідних видів рослин у складних гірських ландшафтах Карпатського національного природного парку, де поєднання висотної поясності, різноманіття оселищ і природних та антропогенних коридорів поширення створює підвищені ризики їх натуралізації та експансії. Метою статті було створення стандартизованого опитувальника для уніфікації процесу фіксації даних про інвазійні види, забезпечення коректної геопросторової прив'язки спостережень та створення передумов для їх подальшого просторового аналізу у середовищі ArcGIS. Форма, розроблена в ArcGIS Survey123, охопила широкий спектр екологічних, біологічних та абіотичних параметрів, зокрема дані про таксономічну приналежність виду, проективне покриття, онтогенетичний стан популяції, характеристики ґрунту, мікрокліматичні показники та рівень антропогенного впливу. Запропонований підхід дозволяє підвищити точність моніторингу інвазійних видів та інтегрувати отримані дані у загальнонаціональні бази спостережень. Конструкція опитувальника базувалася на принципах максимальної сумісності з подальшим аналізом у географічну інформаційну систему та публікацією у глобальних репозиторіях біорізноманіття. Такий підхід відкриває можливість для порівняльних аналізів, злиття з громадянськими спостереженнями і використання даних при оцінюванні ризиків і пріоритезації реагування на інвазії. Методично опитувальник охопив вісім логічних секцій: (1) загальні відомості та геоприв'язка; (2) опис ділянки з класами типів екосистем відповідно до сучасної глобальної типології; (3) характеристика популяції інвазійного виду, включно з площею, щільністю, віковою структурою, динамікою; (4) онтогенетичний спектр з кількісним обліком по стадіях; (5) ґрунтові й мікрокліматичні показники з можливістю польового визначення кислотності та вологості; (6) загрози й пошкодження рослин і екосистем; (7) ґрунтова підстилка як потенційний банк насіння; (8) додаткові коментарі та фотодокументація. Передбачено збір фотознімків різних ракурсів, їх завантаження з просторовою прив'язкою до записів та формування повного цифрового сліду для подальшого аудиту якості

Ключові слова: фітоінвазії; моніторинг; чужорідні види біоти; оселища; біорізноманіття; екосистеми; природно-заповідний фонд

Received: 31.08.2025 Revised: 19.01.2026 Accepted: 26.02.2026 Published: 07.04.2026

BIOLOGICAL SYSTEMS: Theory and Innovation

ISSN 2706-8382
e-ISSN 2706-8390

Journal homepage: https://bioscience.com.ua/en
Vol. 17, No. 1, 2026, 25-35

UDC 632.634.791.937 (477.75)

DOI: 10.31548/biologiya/1.2026.25

Current state of research into leaf-miner moths (Gracillariidae Stainton, 1854) and their feeding habits in relation to tree plantations in Kyiv

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Abstract. The most common plant-feeding insects in the dendrofauna of populated areas, which are prone to mass reproduction, are leaf-miner moths (Gracillariidae Stainton, 1854). The current state, distribution, and reproduction of leaf-miner moths are poorly understood. The purpose of the study was to identify and evaluate species of phytophagous leaf-miner moths in the woody plants of Kyiv. The study of the biodiversity of leaf-miner moths was conducted during 2023-2025 in the city of Kyiv: botanical gardens, parks, squares, streets with woody plants. The presence of leaf-miner moths was assessed visually by the presence of blotches on tree leaves. For this purpose, 100 leaves were collected from the control tree and taken to the laboratory to determine and identify the moth species. The species affiliation of leaf-miner moths was estimated using entomofauna determinants. The occurrence index (P_i) and the dominance index (D_i) were used to characterise the leaf-miner moth species complex. The study identified and described 20 species of leaf-miner moths of the family Gracillariidae (Lepidoptera), which are trophically associated with more than 49 species of host tree plants. These were mostly oligophages. Analysis of the settlement of various species of leaf-miner moth on woody plants in Kyiv showed the following: the highest level of available blotches on the leaves of the plants under study was noted in botanical gardens – 95% of 20 species of leaf-miner moths and parks – 75%. The presence of blotches on the leaves of the studied street trees was observed in 40% of the total number of leaf-miner moth species assessed. According to the indices of occurrence and dominance, it was determined that in the studied locations of woody plants in Kyiv, the dominant ones were *Cameraria ohridella* (95.12%), *Phyllonorycter populifoliella* (83.24%) and *Gracillaria syringella* (53.23%). The results of the study were of scientific value for investigating the biocoenotic relationships between phytophages and

Suggested Citation:
Lisovyy, M., Dulin, A., & Tsurkan, R. (2026). Current state of research into leaf-miner moths (Gracillariidae Stainton, 1854) and their feeding habits in relation to tree plantations in Kyiv. *Biological Systems: Theory and Innovation*, 17(1), 25-35. doi: 10.31548/biologiya/1.2026.25.

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food plants in urban phytocoenoses and of practical importance for the selection of tree species resistant to blotching phytophages used in urban landscaping

Keywords: phytophage; invasive species; monitoring; occurrence index; host plants; abundance

INTRODUCTION

Landscaping of urban settlements, especially megacities, plays an important role in cleaning the air, soil, and creating favourable conditions for recreation of the population. F.-F. Li *et al*. (2024) identified the presence, mass reproduction, and feeding of phytophagous insects as factors contributing to the decline in the physiological, ornamental, and aesthetic qualities of woody plants. A. Ricciardi *et al.* (2021) called the problem of uncontrolled expansion of adventitious pests too urgent, which belongs to one of the most acute environmental problems at the global level, since urban green spaces are the most susceptible to the penetration and further spread of invasive species. In particular, this is facilitated by the import and use of new plant species, the emergence of new ecological niches, a favourable microclimate for the development of phytophages, etc. The same authors, A. Ricciardi *et al.* (2021), noted in their paper that invasive species pose a significant threat to tree stands in cities and forests. H.E. Roy *et al.* (2023) noted that biological invasions are recognised as a growing problem worldwide, with significant environmental, economic, and socio-cultural impacts. O.V. Seliutina *et al.* (2020) noted that in the tree stands of large cities, the dominant and harmful arthropod species are those that have recently begun to reproduce en masse, namely the leaf-miner moths (Gracillariidae Stainton, 1854). The number of species of leaf-miner moths and their population is increasing with each growing season, posing a threat of damage and a reduction in the ornamental value of tree plantations, and environmental pollution from their excrement, which causes allergic reactions in humans. C. Diagne *et al*. (2021) concluded that biological invasions of alien species pose a global threat to biodiversity and human well-being. However, as stated by F.-F. Li *et al*. (2024), governance measures remain largely fragmented across different scales, sectors, and boundaries. Therefore, controlling biological intrusions and their impact requires more than isolated interventions: a globally coordinated system based on scientific information, political will, and public involvement.

The research by I.A. Zaitseva & I.A. Lazariev (2024) noted that in Ukraine, more than two dozen blotching phytophagous insects of the family Gracillariidae have been recorded on the trees of urban areas. Furthermore, L. Vagaliuk *et al.* (2024) noted that the appearance and reproduction of these insect species increased in 2004-2024. For the most part, these are invasive species: *Phyllonorycter platani* (Staudinger, 1870), *Phyllonorycter issikii* (Kumata, 1963), *Cameraria ohridella* (Deschka & Dimic, 1986). M. Zhussip *et al.* (2023) noted that alien species, having entered a new territory in favourable conditions for their development and reproduction, with a sufficient amount of food resource and, importantly, the absence of natural entomophages, multiply extremely rapidly in large numbers, expanding their range and thereby causing significant damage to local and national budgets. C. Diagne *et al.* (2021) in their paper showed that total recorded invasion costs have reached at least USD 1.288 trillion over the past few decades (1970-2017), with an average annual cost of USD 26.8 billion. Moreover, they estimated that the average annual cost could reach USD 162.7 billion. The researchers pointed out that these costs are underestimated, there is no slowdown in the pace, but on the contrary – there is a constant threefold increase over a decade. Furthermore, D.F. Sax *et al*. (2022) noted that among invasive insects, the spread of adventive phytophagous insect species – that is, insects that feed on the photosynthetic apparatus of plants – is becoming increasingly significant; resulting in leaf damage, which significantly affects the physiological condition of plants, leads to their weakening, and repeated damage – to their death.

As a result of the analysis of sources on the selected problem, it was found that the current state, distribution, and reproduction of leaf-miner moths are insufficiently investigated, which was the goal of this study. Thus, the investigation of the biology and ecology of invasive species of phytophagous insects in the conditions of Ukraine is an urgent task. Control of the appearance and reproduction of alien entomofauna species and prevention of the establishment of insect colonies in urban ecosystems of woody plants is possible through constant environmental monitoring and thorough data analysis. The purpose of the study was to identify and investigate species of leaf-miner moth, which are phytophages of woody plants in the city of Kyiv.

MATERIALS AND METHODS

The study of the biodiversity of leaf-miner moths (Gracillariidae Stainton, 1854) was conducted during the growing seasons of 2023-2025 at 10-day intervals across 17 study sites in Kyiv: the M.M. Hryshko National Botanical Garden of the NAS of Ukraine, the National University of Life and Environmental Sciences (NULES) of Ukraine Botanical Garden, and park and street plantings, including Holosiivskyi Park, Kurenivskyi Park, Dubky Park (Syretskyi Hai), Peremoha Park, Natalka Park, Heydar Aliyev Square, and Holosiivskyi Avenue, Druzhby Narodiv Boulevard, Beresteiskyi Avenue, Taras Shevchenko Boulevard, Lesia Ukrainka Boulevard, and the following streets: Khreshchatyk, Saksahanskoho Street, Bohatyrska Street, Olena Teliha Street, and Vyshhorodska Street. A total of 147 model trees representing different species were surveyed (3 model trees per species: 49 × 3), including: small-leaved linden (*Tilia cordata* Mill.), horse chestnut (*Aesculus hippocastanium* L.), oriental plane (*Platanus orientalis* L.), white poplar (silver) (*Populus alba* L.) and european aspen (*Populus tremula* L.), black locust (*Robinia pseudoacacia* L.), silver birch (*Betula pendula* Roth.), black alder (*Alnus glutinosa* (L.) Gaerth.), scots pine (*Pinus silvestris* L.), silver maple (*Lactinium saccharinum* (Wieri), wych elm (*Ulmus glabra* Huds.), large-leaved linden (*Tilia platyphyllos* Scop.) etc. The objects of the study were leaves of woody plants (100 from each model tree) with the presence of blotches, which were marked primarily visually. Leaves from the test trees were selected at random, placed in paper bags, labelled with the location, tree species and date, and transferred to the laboratory for the identification and analysis of the species classification of the leaf-miner moths. The material was collected according to standard methods of inspection, detection, fixation, and determination of blotching dendrophagous insects according to methods by V.L. Meshkova & S.V. Nazarenko (2012) and S.V. Stankevych & S.V. Gornovska (2022). The identification of blotches from leaf-miner moth on the leaves of food plants was carried out using the method developed by the Spanish entomologist C. Lopez-Vaamonde *et al.* (2021) and determinants of entomological biodiversity (Ellis, n.d.). The names of leaf-miner moths were compared according to the electronic catalogue "Fauna Europa" (De Jong, 2024). To determine the population and number of phytophages of woody plants, 100 leaves were selected randomly from the control tree in June-August with an interval of 7-10 days (Silchuk *et al.*, 2016; Lesovoy *et al.*, 2020). The task of the research was not to determine the stage of development of leaf-miner moths, but only the presence of phytophagous blotches and the emergence of a new habitats. The sex of leaf-miner moth species was determined using the method by O.I. Silchuk *et al.* (2016). The occurrence Index (P_i) and the dominance index (D_i) were used to characterise the leaf-miner moths complex. The occurrence index was determined by the ratio of the number of samples (n_l), in which leaf-miner moth were detected (regardless of the number of individuals in the sample) to the total number of samples (N):

$$P_i = (n_I / N)\ 100\%. \qquad (1)$$

The dominance index was determined by how much this species dominated over other identified species both by the frequency of occurrence and the number of individuals in the focus:

$$D_i = P_i N_i / N_s, \qquad (2)$$

where P_i – occurrence of the species, N_i – number of individuals of another species, N_s – total number of individuals.

Identification of leaf-miner moths was carried out according to entomofauna determinants (Lisovoy *et al.*, 1999; De Prins & De Prins, n.d.). The names of insects were given according to the electronic catalogue "Fauna Europaea" (De Jong, 2016). The food plants of the studied species of entomological biodiversity were listed in accordance with the methods described in the National Catalogue of Biotopes of Ukraine and the Classification of Shrub Biotopes of Ukraine (Didukh, 2017; Kuzemko *et al.*, 2018).

RESULTS AND DISCUSSION

Between 2023 and 2025, the phytosanitary condition of tree stands in various locations across Kyiv was investigated for the presence of leaf-miner moth larvae in the foliage (districts: Holosiivskyi, Shevchinkyi, Darnytskyi, Pecherskyi, Obolonskyi, Podilskyi, Dnipro, Solomianskyi, Sviatoshynskyi). During the study period, the taxonomic composition of leaf-miner moths of the family Gracillariidae (Lepidoptera) was identified and recorded in the wooded areas of the city of Kyiv, which are trophically connected with 49 types of food plants. The identified species are listed in Table 1.

Table 1. Species composition of leaf-miner moth (Gracillariidae) that are trophically associated with their host tree species

Species composition, quantity	
Phytophages	**Woody host plants**
Caloptilia semifascia (Haworth, 1828)	*Acer negundo* L., *Acer platanoides* L., *Acer campestre* (L.)
Caloptilia rufipennella (Hùbner, 1796)	*Acer platanoides* L., *Acer saccharinum* L.
Cameraria ohridella (Deshka & Dimic, 1986)	*Aesculus hippocastanum* L.
Gracillaria syringella (Fabricius, 1794)	*Syringa vulgaris* L., *Symphoricarpus albus* (L.) Blake, *Euonymus* sp., *Fraxinus excelsior* L., *Ligustrum* sp., *Forsythia* sp., *Jasminum fruticans* L.
Parectopa robiniella (Clemens, 1863)	*Robinia pseudoacacia* L., *R. pseudoacacia* L. 'Pyramidalis', *R. pseudoacacia* L. 'Unifolia', *R. viscose* Vent.
Phyllocnistis labyrinthella (Bjerkander, 1790)	*Populus alba* L., *P.* × *berolinensis* (K. Koch) Dipp., *P.* × *canescens* (Ait.) Smith, *P. italica* (Du Roi) Moench, *P. nigra* L., *P. simonii* Carr.
Phyllonorycter acerifoliella (Zeller, 1839)	*Acer saccharinum* L.
Phyllonorycter agilella (Zeller, 1846)	*Ulmus glabra* Huds.
Phyllonorycter blancardella (Fabricius, 1781)	*Malus* Mill.
Phyllonorycter cerasicolella (Herrich-Schaffer, 1855)	*Cerasus vulgaris* Mill. (*Prunus cerasus* L.)
Phyllonorycter coryli (Nicelli, 1851)	*Coryllus avellana* L.
Phyllonorycter emberizaepenella (Bouche, 1834)	*Lonicera tatarica* L., *Lonicera xylosteum* L., *Symphoricarpos albus* L.
Phyllonorycter guercifoliella (Zeller, 1839)	*Quercus robur* L.
Phyllonorycter issikii (Kumata, 1963)	*Tilia cordata* Mill., *T.platyphyllos* Scop.
Phyllonorycter platani (Staudinger, 1870)	*Platanus orientalis* L., *P. acerifolia* (Ait.) Wild.
Phyllonorycter populifoliella (Treitschke, 1833)	*Populus italica* (Du Roi) Moench, *P. nigra* L.
Phyllonorycter salicicolella (Sircom, 1848)	*Populus nigra* L., *Salix alba* L.
Phyllonorycter sorbi (Frey, 1855)	*Sorbus aucuparia* L., *Cotoneaster* Medik., *Crataegus* L., *Malus* Mill., *Padus* Mill., *Prunus* L.
Phyllonorycter ulmifoliella (Hùbner, 1817)	*Betula pendula* Roth., *Betula pubescens* Ehrh.
Phyllocnistis labyrinthella (Bjerkander, 1790)	*Populus alba* L., *P. tremula* L.
20	**49**

Source: developed by the authors

As a result of the research, it was determined that the identified phytophagous species were divided into groups according to their trophic specialisation: polyphages – 2 species, oligophages – 12 species, monophages – 6 species. Analysis of the distribution of leaf-miner moths in various types of tree stands in Kyiv showed that most of the species were recorded in the M.M. Hryshko Botanical Garden of the NAS of Ukraine and the Botanical Garden of the NULES of Ukraine (19 species), and in parks of varying sizes (Holosiivskyi, Kurenivskyi, and Peremoha) – 15 species. An analysis of the distribution of various species of striped moths on woody plants in Kyiv was carried out, and the following was observed: the proportion of leaves infested on the trees in botanical gardens accounted for 95% of the total number of species (20) of leaf-miner moths identified, and in parks – 75%. In the street tree stands under study, which were used for landscaping, the presence of the blotches by leaf-mining moths accounted for only 40% of the total number of blotching insects. The difference in the number of identified leaf-mining moth species in botanical gardens and parks, according to the authors, may lie in the species range of woody plant species. The aforementioned botanical gardens include hundreds of species and subspecies of woody plants, whereas this diversity is considerably lower in the parks mentioned. For example, the dominant tree species in Holosiivskyi Park is European hornbeam (*Caprilus betulus* L.), in Kurenivskyi Park it is small-leaved linden (*Tilia cordata* Mill.), and in Peremoha Park the dominant species are black alder (*Alnus glutinosa* (L.) Gaerth.), poplar (*Populus* L.), and scots pine (*Pinus silvestris* L.). 8 species of leaf-mining moths were found on woody plants growing along roads with heavy traffic (Table 2). Noise and gas pollution from car exhaust gases play the role of a repellent barrier, as evidenced by the low number of phytophagous insects (8 species).

Table 2. Distribution of identified leaf-mining moth species by types of plantings in Kyiv (2023-2025)

Type of phytophage	Tree stands		
	Botanical gardens	Parks and squares	Streets
Caloptilia semifascia	+	+	-
Caloptilia rufipennella	+	+	-
Cameraria ohridella	+	+	+
Gracillaria syringella	+	+	-
Parectopa robiniella	+	+	+
Phyllocnistis labyrinthella	+	+	+
Phyllonorycter acerifoliella	+	+	-
Phyllonorycter agilella	+	+	-
Phyllonorycter blancardella	+	+	-
Phyllonorycter cerasicolella	+	+	+
Phyllonorycter coryli	+	-	-
Phyllonorycter emberisaepenella	+	-	-
Phyllonorycter guercifoliella	+	-	-
Phyllonorycter issikii	-	+	+
Phyllonorycter platani	+	-	+
Phyllonorycter populifoliella	+	+	+
Phyllonorycter salicicolella	+	+	-
Phyllonorycter sorbi	+	-	-
Phyllonorycter ulmifoliella	+	+	-
Phyllocnistis labyrinthella	+	+	+
Total:	**19**	**15**	**8**

Note: “+” – detected; “–” – species not present
Source: developed by the authors

An ecological analysis of the results (Table 2) on the presence of mottled moths in different stations was carried out and it was determined that botanical gardens, parks, squares, and tree stands along the streets are different in species abundance and number of tree species, which was reflected in trophic relationships with phytophagous insects. Botanical gardens and parks contain the full range of tree host plants for leaf-mining moths; the trees are more remote and protected from anthropogenic and abiotic factors that affect the insect fauna, and consequently 19 out of the 20 species of the leaf-mining moth insect complex have been recorded. Completely different features in tree stands along the streets are a significant impoverishment of the species composition of plants (1, less often 2 species), which is a limiting factor in the development of diversity of all 20 species of leaf-mining moth. The following species have been recorded, mainly monophagous species of tree plants: *Cameraria ohridella* (Deshka & Dimic, 1986), *Parectopa robiniella* (Clemens, 1863) and, less frequently, oligophagous species – *Phyllonorycter issikii* (Kumata, 1963).

According to the calculation of the occurrence indices (equation 1) of leaf-mining moths on woody plants of certain research sites, it was noted that the plants were most frequently damaged by *Cameraria ohridella* – 7.51%, and *Phyllonorycter populifoliella* – 4.52%. Based on the calculations of the dominance indices (equation 2), it was established that in the tree stands of Kyiv, three types of leaf-mining moth were dominant: *Cameraria ohridella*, *Phyllonorycter populifoliella*, *Gracillaria syringella* – 95.12%, 83.24%, 53.23%, accordingly. Two species of leaf-miner moths have been recorded in the tree plantations of Kyiv: *Phyllonorycter platani* (Holosiivskyi Avenue) and *Phyllonorycter emberizaepenella* (Heydar Aliyev Square), which, according to an analysis of the literature for the period 2016-2025, are classified as invasive species in the region under study. Both species are oligophages, which means that there are all prerequisites for their mass reproduction, which can have negative consequences for trophically related woody plants in the research region. The researchers noted that the establishment of a new range for invasive species of leaf-mining moth was determined by a combination of climatic factors, the absence of natural entomophages, and the distribution of woody plants that are trophically linked to the phytophages. For example, the expansion of the range of the Oriental plane (*Platanus orientalis* L.) and London plane *P. acerifolia* (Ait.) Wild. from the southern region to Kyiv contributed to the expansion of the range of *Phyllonorycter platani* (Staudinger, 1870) in this region, although the cause and route of penetration of the invasive phytophage is unknown.

After analysing the distribution of blotching phytophagous insects in other localities and countries, the ambiguity of settlement, and reproduction of invasive blotching insects and their interaction with woody host plants was noted. N. Kirichenko *et al.* (2019) noted that leaf miners are a taxonomically diverse group of endophagous insects. Some of them are harmful in forestry, horticulture, and agriculture, and some have become major invasive species. The researchers also described the biological characteristics of invasive leaf miners of woody plants and listed 12 invasive leaf-mining species found in the study area, belonging to four different orders of insects. For each of them, N. Kirichenko *et al.* briefly described their intrusions, including their pathways, impact and management methods, and ecology. Some species have a significant economic impact as garden pests, such as citrus miner (*Phyllocnistis citrella*), or as pests of ornamental plants, such as the horse-chestnut leaf miner (*Cameraria ohridella*). The researchers noted that the ecological impact of these species should be better studied, especially those that destroy native trees such as the beech leaf weevil (*Orchestes fagi*) in Canada. The presented study covered only leaf-mining moth of the family Gracillariidae and 20 species estimated within Kyiv, which allowed comparing the results obtained with a wider list of invasive leaf miners, given in the paper by N. Kirichenko *et al*. (2019). Comparison of data on the distribution routes of phytophages confirmed the conclusions of these researchers about the need for further studies of the mechanisms of their settlement, and the search for local natural parasitoids as an important biological method for controlling the number of invasive species.

K.K. Holoborodko *et al.* (2021) noted that the complex of invasive phytophagous insect species that can adapt to new living conditions is constantly growing. The ecological effect of their vital activity in the new environment is almost impossible to predict, and as a result, economically undesirable invasions are often observed. The horse-chestnut leaf miner (*Cameraria ohridella*) (Deschka & Dimič, 1986, Gracillariidae) is one of these problematic invasive species in the introduced range of the horse chestnut (*Aesculus hippocastanum* (Linnaeus, 1753), Sapindaceae). The researchers investigated the impact *C. ohridella* on the state of the photosynthetic apparatus of *A. hippocastanum* leaves and studied the effect of caterpillar feeding *C. ohridella* on the functional state and activity of the photosynthetic apparatus of horse chestnut trees. In this study, changes in photosynthetic activity in the leaves of horse-chestnut under the influence of feeding horse-chestnut leaf miner larvae were not analysed. K.K. Holoborodko *et al*. (2021) and literature data allowed comparing them with the results obtained and emphasising the relevance of research aimed at creating forms of *A. hippocastanum* that are more resistant to the enzymatic effects of *C. ohridella* larvae.

I.A. Zaitseva & A.S. Lazariev (2022) in their research for the first time compared the population of walnut (*Juglans regia* L.) and two types of invasive phytophages – *Aceria erinea* (Nalepa, 1891) and *A. tristriata* (Nalepa, 1890) in the city of Dnipro (Ukraine) on 75 trees. The average population of these phytophages was 42.8%. No phytophages were observed in a separate study area (Tunnelna

Balka tract). Further, the researchers noted that the population of *A. tristriata* on the trees *J. regia* was 27.3%, which was higher than the population of *A. erinea* (15.5%) by 11.8%. The average number of galls per leaf of *J. regia* caused by the pest *A. erinea* was 1-6.2, whereas that caused by *A. tristriata* – 19.1-144.2. Further research on the ecology of invasive phytophages *J. regia* is planned. Although the present study did not analyse the population of invasive species on walnut, the results obtained by I.A. Zaitseva & A.S. Lazariev (2022) provide an opportunity to compare their observations with the results obtained in this study and confirm the feasibility of further studies of the ecology of invasive phytophages in wider areas of distribution of the same tree species.

O.V. Seliutina *et al.* (2020) conducted a study and analysed the dynamics of leaf damage, the content of easily soluble proteins, benzidine peroxidase activity, and the composition of acid enzyme isoforms in leaves of *Aesculus hippocastanum* L. under the influence of colonisation and nutrition of the horse-chestnutleaf miner (*Cameraria ohridella*) (Deschka et Dimić) (Lepidoptera, Gracillariidae) and noted that the horse chestnut responds specifically to larval nutrition of *C. ohridella* due to changes in the activity of individual peroxidase isoenzymes, the expression of which varies across the entire spectrum. In horse chestnut leaves with a high level of damage caused by the horse-chestnut leaf miner, the expression of enzyme isoforms in the pH range of 4.08-4.15 is significantly enhanced, which may be regarded as a reliable biochemical marker of the sensitivity of *A. hippocastanum* plants to feeding by the phytophagous insect *C. ohridella.* This study focused on identifying infestations of tree species in Kyiv by the leaf-miner moth in various locations and on identifying the moths themselves, thereby enabling the results to be compared with the biochemical indicators of the reaction of *Aesculus hippocastanum* described in the paper by O.V. Seliutina *et al.* (2020). This comparison indicated the feasibility of further studies of biochemical changes in the leaves of woody plants that occur in response to the nutrition and development of tunnelling phytophagous insects.

V. Tytar *et al.* (2022) associated spread and outbreaks of phytophagous pests with global warming. In addition to economic interest, the species under study may be biological indicators of climate change. As an example, the researchers investigated the locust leaf-miner moth (*Parectopa robiniella* (Clemens, 1863) (Lepidoptera: Gracillariidae), which is actively spreading across the continent. Using a georeferenced record of *P. robiniella* across Europe and the Bayesian additive regression tree (BART) algorithm, the researchers modelled the distribution of the leaf-miner moth. The researchers concluded that, among the factors determining the ecological niche of *Parectopa robiniella* Clemens, 1863, temperature conditions are the key factor facilitating the spread and natural adaptation of this phytophagous insect. Results of the study by V. Tytar *et al*. (2022) regarding the determining role of temperature conditions in the spread and natural adaptation of phytophage are consistent with the observations. A comparison of these findings with the results of the study also indicates that the distribution range of the host plant, which is trophically linked to the pest, plays an important role in the establishment of secondary ranges for invasive species of leaf-miner moths, particularly in the case of monophagous or oligophagous species.

I.M. Shvydenko *et al.* (2021) noted that *Parectopa robiniella* (Clemens, 1863) is an native species of North America that spread to Italy, Slovenia, Croatia, Austria, Slovakia, Romania, Hungary, and Ukraine in the 1980s. *Phyllonorycter robiniella* (Clemens, 1859) is also of North American origin. The leaf-miner moth was found in Switzerland in 1983, France, Germany, Italy in 1988, Austria in 1990, Slovakia in 1992, Slovenia in 1994, Hungary in 1996, the Netherlands in 1999, and Bulgaria in 2003. At present, both Robinia-associated phytophagous species are distributed in 19 European countries. In Ukraine, these species appeared relatively recently and are insufficiently studied. The researchers investigated some bioecological features of *P. robiniella* and *Ph. robiniella* in the conditions of the Kharkiv region: the density of tunnels/leaves, the swarming dynamics, and the state of populations. Bioecological features of these invasive species of blotching phytophages were not analysed in this study, since attention was focused on describing the appearance and development in Kyiv of such types as *Cameraria ohridella* (Deschka & Dimić, 1986), *Phyllonorycter platani* (Staudinger, 1870), *Phyllonorycter issikii* (Kumata, 1963) etc., results for which were obtained in previous studies. Comparison of these data with results by I.M. Shvydenko *et al*. (2021) provided an opportunity to contrast the features of the spread and development of invasive blotching phytophages in different regions of Ukraine.

M. Paterska *et al.* (2025) analysed the phytosanitary status of horse chestnut trees, susceptible and resistant varieties, against *Cameraria ohridella* (Deschka & Dimić) in Europe and noted that this invasive phytophage colonises trees of the genus *Aesculus* every year throughout Europe, causing dark brown damage (tunnels) that gradually cover the surface of the leaves. As a result of the studies, the profiles of volatile organic compounds (VOCs) released by chestnut leaves with different susceptibility to phytophage were compared, and the results of changing the profiles in which the larvae fed were evaluated. The study included susceptible tree species *Aesculus turbinata* and resistant trees *Aesculus glabra* and *A. parviflora* during the three growing seasons, leaf damage and VOC profile were analysed biweekly, from May to September. Leaf damage progressively grew in *A. turbinata* throughout all seasons. VOC profiles differed both qualitatively and quantitatively between trees of favourable and resistant species. Results by M. Paterska *et al*. (2025) on differences in the profiles of volatile organic compounds in susceptible and resistant species of *Aesculus* formed an important basis for comparison with the results of this study, in which such biochemical parameters were not analysed, since this was not part of the tasks set. Comparison of these data with the results obtained highlights the feasibility of further biochemical studies of changes in the leaves of woody plants that occur in response to the nutrition and development of tunnelling phytophagous insects.

Thus, the reasons for the appearance, behaviour, and settlement of new territories by invasive biodiversity species in Europe and Ukraine are not sufficiently studied. Problems of invasive leaf-miner moth species in tree stands in Kyiv are significant and ignoring this issue will be costly in the not-too-distant future. To avoid negative unpredictable consequences, monitoring studies on the state of appearance and development of invasive species in the research region are necessary.

CONCLUSIONS

As a result of research (2023-2025), 20 species of leaf-miner moths were identified on tree stands in Kyiv, which systematically belong to the family Gracillariidae. It was established that the number of studied species of leaf-miner moths, which are trophically related to 49 species of host trees growing in botanical gardens, parks, and along highways in Kyiv, is: in botanical gardens – 95% of the 20 species of leaf-miner moths, parks – 75%, outdoor tree stands – 40% of the total number of estimated species of leaf-miner moth. The analysis of leaf-miner moths by trophic specialisation with woody plants was carried out and it was determined that 6 of them are polyphages, 14 are oligophages, and 3 are monophages. The group of oligophages was found to be the most numerous in terms of trophic specialisation, suggesting that the group of phytophages is potentially best adapted to the new conditions. The invasive phytophagous insects identified as dominant in the tree stands of Kyiv, based on the dominance index, were: *C. ohridella*, *P. populifoliella*, and *G. syringella*, with dominance indices of 95.12%, 83.24%, and 53.23%, respectively. Two species of leaf-miner moths have been recorded in the tree stands of Kyiv: *Phyllonorycter platani* and *Phyllonorycter emberizaepenella*, both of which are classified as invasive species in the study region. To date, it is not known for certain how these alien insect species in the tree plantations of the studied locations in Kyiv first appeared, adapted, and proliferated. There is an assumption that the presence of woody host plants in research sites based on trophic relationships contributes to the reproduction of alien entomofauna species in new biocoenoses (Kyiv, Ukraine).

Thus, given the above, it can be stated that the rate of infestations, the absence of natural entomophages, the ecological plasticity that invader species of the Gracillariidae family are endowed with, and research on the state of their populations, are important for science, namely, the ecology of entomological species. Prospects for further research are associated with an in-depth study of trophic relationships of leaf-miner moths, in particular invasive species, which will allow substantiating and developing effective strategies for regulating their numbers and improving the system of protection of tree stands of settlements from common invasive entomological phytophages of ornamental and landscape crops in the city of Kyiv.

ACKNOWLEDGEMENTS

None.

FUNDING

None.

CONFLICT OF INTEREST

None.

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Сучасний стан досліджень молей-строкаток (Gracillariidae Stainton, 1854) та їх трофічна спеціалізація з деревними насадженнями м. Києва

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Анотація. Найбільше розповсюдженими комахами-фітофагами дендрофауни населених пунктів, які набувають масового розмноження, є молі-строкатки (Gracillariidae Stainton, 1854). Сучасний стан, розповсюдження та розмноження молей-строкаток недостатньо вивчені. Метою досліджень було виявлення та оцінювання видів молей-строкаток, які є фітофагами деревних рослин в м. Києві. Дослідження біорізноманіття молей-строкаток було проведено впродовж 2023-2025 років у м. Києві: ботанічні сади, парки, сквери, вулиці, де є деревні рослини. Наявність молей-строкаток оцінювали за наявністю мін на листках дерев візуально. Для цього відбирали по 100 листків з контрольного дерева і переносили в лабораторію для визначення та ідентифікації видів молей. Видову приналежність молей-строкаток оцінювали за допомогою визначників ентомофауни. Для характеристики видового комплексу молей-строкаток використовували індекс трапляння (P_i) та індекс домінування (D_i). В результаті досліджень виявлено і визначено 20 видів молей-строкаток родини Gracillariidae (Lepidoptera), які трофічно пов’язані з більш як 49 видами деревних рослин-господарів. В основному це олігофаги. Аналіз заселення різними видами молей-строкаток деревних рослин у м. Києві показав наступне: найвищий рівень наявних мін на листках досліджених рослин відмічали в ботанічних садах (95 % з 20 видів молей-строкаток) і парках (75 %). Наявність мін на листках досліджуваних дерев вуличних насаджень відмічена на рівні 40 % від загальної кількості оцінених видів молей-строкаток. Згідно індексів трапляння та домінування визначено, що на досліджуваних локаціях деревних рослин Києва домінуючими є *Cameraria ohridella* (95,12 %), *Phyllonorycter populifoliella* (83,24 %) та *Gracillaria syringella* (53,23 %). Результати дослідження мають наукову цінність для вивчення біоценотичних зв’язків між фітофагами та кормовими рослинами в урбофітоценозах і практичне значення для вибору стійких до мінуючих фітофагів деревних порід, що використовуються в міському озелененні

Ключові слова: фітофаг; інвазійний вид; моніторинг; індекс трапляння; рослини-господарі; чисельність

Received: 19.09.2025 Revised: 27.01.2026 Accepted: 26.02.2026 Published: 07.04.2026

BIOLOGICAL SYSTEMS: Theory and Innovation

ISSN 2706-8382
e-ISSN 2706-8390

Journal homepage: https://bioscience.com.ua/en
Vol. 17, No. 1, 2026, 36-51

UDC 631.95:504.06

DOI: 10.31548/biologiya/1.2026.36

Methodological approaches to assessing anthropogenic impact on agrocenoses

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Abstract. The relevance of the study is conditioned by the increase in the anthropogenic load on agrocenoses caused by the intensification of agriculture, the increase in chemical load and changes in the structure of land use. At the same time, these factors change the soil state, structure of the accompanying phytocenosis and the state of the biotic communities. This study aimed to systematise and compare the existing methodological approaches to assessing the effect of human impact on agrocenoses in the framework of the "pressures – state – impact" concept. A thematic synthesis of publications for 2019-2025 was carried out; sources that had clearly formulated indicators and an explicitly described procedure for their calculation were selected for the analysis. The generalisation was carried out according to the following analytical blocks: soil, biota, vegetation and geospatial tools. The results show that the most reliable estimates were obtained using a complex application of physical, chemical and biological indicators of the soil state; single-dimensional indicators lose their sensitivity to the management impact and do not reflect systemic changes. Bioindication based on enzymatic activity, microbial processes and reaction of soil fauna was found to be effective for early diagnostics of the degradation process and toxicological danger before irreversible changes in physico-chemical properties occur. Indices of plant diversity and dominance, as well as functional indicators, respond to the selective impact of intensification of agriculture and herbicide load, reflecting the simplification of communities and the change in the species composition. It was found that the application of geographic information systems and remote sensing of the Earth increases the comparability of estimates due to the possibility of spatial and temporal extrapolation, revealing within-field heterogeneity and risk zoning. Integral indices contribute to improving the comparability of territories; however, their application requires a harmonised interpretation of the direction of indicators, transparent weighting and an explicitly

Suggested Citation:
Naumovska, O., Berezhniak, Ye., & Aponiuk, D. (2026). Methodological approaches to assessing anthropogenic impact on agrocenoses. *Biological Systems: Theory and Innovation*, 17(1), 36-51. doi: 10.31548/biologiya/1.2026.36.

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described uncertainty consideration. The results confirmed that a comprehensive approach that combines soil, biotic, plant and geospatial indicators is the most effective for monitoring, comparison of territories and decision-making for agrotechnical measures to minimise the negative environmental effect of agroproduction

Keywords: intensification; soil health; bioindication; landscape heterogeneity; remote sensing

INTRODUCTION

The agrocenoses are among the most strongly modified biotic systems in which the structure of the biota, the directions of the flow of matter and energy, and the type of ecosystem functions are determined not only by natural, but mainly by management conditions. The anthropogenic factor in the cultivation of crops and keeping of livestock is represented by a complex of directed technological impacts, chemical, mechanical and spatial, that change the physico-chemical properties of the soil, the structure of the phytocenosis and the state of the other biotic components functioning in it. In intensive technologies, the growth of productivity is usually provided by growing inputs of resources, the concentration of impacts, and increasingly complicated environmental costs. This tendency was clearly demonstrated in the analysis of the drivers, consequences, and alternatives of intensification in the dairy sector carried out by N. Clay *et al*. (2020). For this reason, the methodological estimation of the anthropogenic impact involves not only the description of the separate factors, but also the development of integral approaches to their quantitative measurement and comparison at the levels of the field, farm, and agrolandscape. One of the main methodological problems arises from the nonlinearity of the ecological reactions, when the growth of the pressure does not lead to commensurate changes in the indicators of the state; in certain situations, the system may move to another mode of functioning. The problem of threshold transitions in ecosystem services of agrolandscapes and the likelihood of intensification inducing “tipping points” was grounded in the research of S.C. Watson *et al*. (2021). It was pointed out that there is a need not only to reveal the average changes, but also the critical changes in the development trajectories. In this situation, indicator systems narrowed down to separate indicators lose their informative value, while systems that formalise cause-and-effect relationships and allow distinguishing between the driving forces, pressures, state, and impacts gain value. The Driving Force – Pressure – State – Impact – Response methodological framework, which has been elaborated for the evaluation of land, soil and water resources in agriculture, was systematised by A. Bhaduri *et al*. (2022) to provide the grounds for comparative analysis and for transforming diagnostic results into management decisions.

In the majority of agrocenosis assessments, soil has retained its position as the key object because, on the one hand, it performs a productive function, and, on the other hand, it serves as an accumulator of risks of degradation and pollution. The development of systems of indicators of soil health in relation to long-term fertilisation was shown by X. Li (2025), who proposed the construction of sets of indicators and rules for their aggregation for typical agricultural territories in conditions of long-term application of fertilisers. Such investigations are necessary to ensure comparability, as long-term fertilisation changes not only the nutrient regime but also microbiological and structural properties of soils, which, in their turn, affect the reaction of vegetation and biotic communities. The national dimension of methodological provision for risk analysis has been expanded through a discussion of methods for evaluating environmental risks proposed by A. Lishchuk *et al*. (2023b). According to the authors of that study, it is necessary to formalise the procedures for analysis and to strictly distinguish risks from their sources and from conditions of their manifestation. Therefore, the coordination of soil indicators with risk analysis has been viewed as a step from the level of local observations to the level of inferences significant for managerial decisions.

The anthropogenic load on agrocenoses comes not only from external but also from internal sources of chemical stress that affect not only the state of the soil but also the interaction of biotic components and the environmental safety of agrolandscapes. An ecotoxicological assessment of the effect of landfills for solid communal waste on neighbouring agrocenoses was made by P.V. Pysarenko *et al*. (2022), who showed that agrosystems can be exposed to essential loads not related to agricultural technologies and that those loads should be integrated into the general framework for evaluation. Among technological factors, the pesticide burden is one of the most powerful, because its impact can be implemented in the form of both acute toxic action and selective effects on the biota and the structure of communities. Regional studies on the environmental risk of pesticide loads in agrocenoses of predecessors of cereal crops were presented by A. Lishchuk *et al*. (2023a); the authors emphasised the need for integration of data on the application of pesticides with state indicators and disturbance indicators. A more general synthesis of the approaches to the management of pesticide risk in agrocenoses stressed the importance of indicators capable of diagnosing early negative shifts and of spatial heterogeneity of the impacts that would provide a basis for management decisions not built on the observance of the standards.

The analysis of the plant component is methodologically important not only as a characteristic of the impacted object but also as a tool for fixation of the system response of the agrocenosis to management impacts. The approach to the use of functional indicators for the explanation of the anthropogenic changes in the species composition was justified by L. Bonilla-Valencia *et al*. (2020). The authors showed that the interpretation of changes becomes more informative if based on functional traits related to disturbance and stress rather than on taxonomic inventories. For the transition from the field level to the agricultural landscape level, the quantitative description of the spatial structure is of principal importance because it defines ecological connectivity, potential of recovery, and stability of biodiversity. The quantitative evaluation of agrolandscape heterogeneity by land-cover structure metrics was shown by N.P. Seidl & M. Golobič (2020). The authors offered methodological instruments for comparison of territories and for consideration of the landscape context in the interpretation of “state” indicators. This created a demand for a methodological complex capable of integrating technological impacts, soil and biotic processes, plant indicators, and spatial structure of the environment within a single conceptual system of evaluation.

This study aimed to incorporate modern methodological tools for evaluating anthropogenic effects on agrocenoses into the “pressures – state – consequences” analytical framework, ensuring the comparability of assessments at sites and spatial scales. To implement this aim, the following tasks were formulated: to generalise indicator-based approaches for assessing the state of the soils and environmental risks at different types of impact; to describe approaches to identifying changes in the structure of the plant community and landscape heterogeneity as modifying environmental consequences; to justify the rationale for integrating indicators into causal schemes, convenient for territorial comparisons and decision-making.

MATERIALS AND METHODS

The analysis of methods for assessing anthropogenic effects on agrocenoses was carried out as a narrative (thematic) review with elements of evidence synthesis (scoping review) aimed at systematising and comparing indicator-based approaches for the soil component, plant communities and associated biological components, primarily soil biota. The analysis of methods for assessing anthropogenic effects on agrocenoses was carried out as a thematic synthesis of modern approaches, including bioindication, ecological indices of plant communities and geospatial methods, with a focus on the soil component, plant communities and related biological elements, primarily soil biota. In the context of agrocenoses, anthropogenic effects were understood as a comprehensive impact of controlled agrotechnical measures and land-use spatial transformations leading to changes in the physical-chemical and biological state of the soil, the structure of accompanying vegetation and the functioning of biotic communities. The analysis was structured according to the “pressures – state – consequences” scheme, which allowed associating specific drivers of agricultural intensification with measurable indicators of the system state and indicators of ecological consequences.

The analysed materials consisted of publications from 2014 to 2025, with a focus on the latest research published from 2019 to 2025, during which the intensification of the development of indicator systems for soil health, geospatial methods, and remote sensing occurred. The search of literature was carried out in the main bibliographic and full-text bases: Scopus, Web of Science Core Collection, PubMed, and Google Scholar. In addition, methodological and technical documents of normative character were analysed. Among them were the materials of the European Union (EU) on monitoring soil (Directive (EU) 2025/2360, 2025); metadata for statistical data from Eurostat (n.d.); and documentation for Earth observation programmes for agriculture (Sen4CAP, 2017). Requests were formed in Ukrainian and English and combined into thematic blocks: agrocenoses and land-use intensity; technological pressures; soil indicators; plant communities; geospatial methods; vegetation indicators; and modelling. The analysis included articles, reviews, systematic reviews, and methodological materials that contained an explicit description of the indicator, procedure for its calculation, or the presentation of the required data. Materials not related to agrocenoses, those without methodological descriptions, and declarative articles were excluded. The preference was given to studies whose methods could be compared in terms of the scale of application, the type of data (field, monitoring, satellite or model), and the ability to integrate into comprehensive indices.

The state of soils was considered as a combination of physical, chemical and biological indicators; single-dimensional assessments do not allow for the identification of systemic changes. The greatest attention was paid to indicators of soil compaction and structure, content of organic matter or organic carbon, acidity, nutrient regime, and, if necessary, salinisation and pollution. The biological component of soil was represented through indicators of microbial activity, enzymatic tests and indicator groups of soil fauna, which reflect the functional consequences of agricultural pressures and respond sensitively to the intensity of tillage, chemical loads and deficiencies of organic matter. The selection of bioindicators was interpreted with regard to the fact that their values depend strongly on season, moisture conditions and soil type. For this reason, the synthesis emphasised the need to compare data obtained under comparable natural conditions.

The evaluation of the effect on plant communities was represented by the analysis of accompanying flora as an indicator of disturbance and selection pressure, as well as by applying ecological indices of diversity and dominance. It was shown that these indicators allow tracing the simplification of communities, changes in species composition, and an increase in the proportion of dominant species in response to intensive technological impacts. At the scale of the agricultural landscape, the role of semi-natural elements and spatial mosaics was also considered, as these factors contribute to the conservation of biodiversity and the buffering of agricultural pressures. For comparing between fields, crops and regions, the articles reviewed here consistently used species richness and community diversity indexes, such as the Shannon diversity index and the Simpson dominance index. Their use ensured the comparability of assessments under differing levels of technological pressure. New analytical techniques have been developed in combination with Geographic Information System (GIS) and remote sensing to describe spatially and temporally the status of the crops and the structure of the land cover. Remote sensing data were used as a proxy for providing indicators of seasonal, phenological and intra-field variability, which allowed the extrapolation of point measurements to larger areas. Geoinformation techniques were used to combine point data with mapping data layers, to consider the landscape context and to define risk areas for degradation.

Special emphasis was placed on aggregated indices, which allow the combination of multiple variables into a composite index for soil, biota and vegetation, making possible the comparison between areas or between different time periods. The discussion emphasised that aggregating indexes requires consensus on the direction of the indexes' interpretation, a clear weighting procedure and a clear uncertainty analysis in order to prevent the index results from depending too heavily on the mathematical framework. The last part of the review was related to the comparison of methods used to predict the response of management practices, where models were used to evaluate scenarios of change in nutrient cycling, soil erosion, carbon cycle, and other environmental impacts. It was concluded that the combination of monitoring data, remote sensing indexes and model outputs improves the confidence of the results and the practical use of the methods for the sustainable use of natural resources, biodiversity and minimisation of the environmental impact of agricultural practices. Limitations of the study consisted of the fact that the results are based on a qualitative comparison between articles and not on a common dataset of field measurements. In addition, the diversity of methods, scales of study and environmental conditions among the studies considered here limits the extrapolation of critical levels and ranges to other regions.

RESULTS AND DISCUSSION

Structuring causal relationships within the framework of "pressures – state – consequences"

The synthesis of selected studies showed that most replicable and analytically powerful approaches were those where human impact on agrocenoses was described through an analytically clear and sequentially consistent differentiation between technological pressures, the state of system components and ecological consequences. This three-level logic was used both as a tool for results structuring and as a tool for the control of typical methodological mistakes in causal inference in agroecological studies. The synthesis revealed that, in the absence of a distinct separation between "pressure" and "state", most indicators become non-informative: the same indicator starts to be read simultaneously as cause and effect. This ambiguity leads to circular readings and overestimates the apparent logic of inferences. This is why causally structured frameworks are the only ones ensuring the comparability of results between studies, territories and time periods.

In the synthesis, pressures in agrocenoses were understood as managed regimes of intensity, not as particular technological interventions. The intensity of tillage, agrochemical loads, simplification of crop rotations and land-use changes were found to have a cumulative nature and often synergise. This is why the same doses or technologically similar measures can cause different effects: they depend not only on the "dose" but also on the "exposure" defined by the toxicological properties of substances, transport conditions, frequency of applications and landscape-soil situation. As a result, in methodologically valid studies, the pressure was not reduced to its mere application but was characterised by frequency, dose, seasonality, application technology and landscape-soil conditions.

The DPSIR scheme (Driving forces – Pressures – State – Impact – Response) appeared to be an adequate tool for ordering such causal chains, since it enables the separation of levels of description and helps to maintain logical coherence in the identified relationships. In agroecosystem applications, DPSIR formalises the connection between agricultural intensification and changes in ecosystem functions by aligning driving forces and pressures with sets of state and impact indicators (Moss *et al.*, 2021). The practical significance of this scheme is that it makes it possible to identify "gaps" in the causal chain: if any pressure has no state indicators or they are weakly connected to the impact mechanism, then the subsequent reasoning about the consequences is methodologically incomplete.

When evaluating the sustainability of agricultural territories with the help of the DPSIR, the combination of diverse indicators made it possible to create comparable "pressures" and "state" profiles, which allowed the understanding of the differences in the ecological efficiency of management at the level of territorial units (Chen *et al.*, 2024). It turned out that high values of pressure indicators should not be considered as an unambiguous sign of degradation in the absence of confirmation by state indicators, whereas satisfactory state parameters do not exclude risk in conditions of accumulation of pressures or in the presence of a delayed reaction of the soil-biotic complex. As a result, the most reliable estimates were those that took into account the delay, nonlinearity and the variable buffer capacity of systems depending on the soil and landscape situation. In the works systematising the interaction of agriculture with aquatic ecosystems, the DPSIR logic made it possible to clearly distinguish agricultural pressures from the natural variability of the hydrological regime and to build transparent causal chains between agricultural production and the state of aquatic ecosystems (Troian *et al.*, 2021). This demonstrated the relevance of the agrolandscape scale, because some impacts occur outside the field, as a result of matter transfer and changes in other environmental compartments. Hence, a causally structured approach was most effective when field-level indicators were combined with indicators of landscape structure and material flux.

That the "pressures – state – impacts" logic is consistent with the policy level of observation was supported by the fact that normative documents on monitoring of soils are focused on systematic observation and comparable characteristics of soil sustainability and degradation that can be compared in dynamics and between territories (Directive (EU) 2025/2360, 2025), which makes the necessity of transparent connection between indicators, specific pressures and expected responses even more obvious. At the same time, it was stressed that striving for comparability does not remove the contextuality, but only increases the requirement for adequate accounting for the natural background, primarily through stratification by soil types and landscape features.

In general, it was found that structuring assessment according to the DPSIR scheme provides methodological reliability (prevents confusion of causes and responses), readability (provides an explanation for the variability of responses under the same technologies) and is the basis for management recommendations (connection of certain management practices with expected changes in state), which are the reasons why causally-oriented schemes remain relevant for integration of soil, biotic and phytometric indicators as well as remote-sensing data into a unified system for diagnostics of anthropogenic influence on agrocenoses.

Dependence of interpretation on soil indicators

The synthesis of approaches to assessing the state of soils showed that modern methods are mainly represented by combinations of physical, chemical and biological indicators, single-dimensional assessments do not embrace system-wide changes and often lose sensitivity to management pressures (Guo, 2021). The analysed publications demonstrate a clear tendency to abandon universal singleindicator solutions in favour of sets of indicators, where each block of indicators corresponds to a specific mechanism of degradation or functional transformation. Physical indicators were most often considered markers of mechanical exposure and structural destruction, primarily compaction and loss of porosity, which directly restricts the infiltration, gas exchange and availability of moisture for the root system. Chemical indicators were found to be more appropriate for estimating long-term changes in the regimes of buffering capacity, acidity, salinity and nutrients, which appeared as a result of fertilisation, elements leaching, amelioration activities and soluble compounds transfer. Biological indicators were considered as a "functional layer" of assessment, because they reflect the intensity of soil processes, the response of the microbial community to changes in the availability of organic matter, and the capability of the soil to maintain a stable biogeochemical cycle.

It was concluded that the main methodological justification for the comprehensive approach was uncertainty in the interpretation of individual indicators in different types of impact. Thus, a decrease in organic matter content or in the content of soil organic carbon can be caused both by intensive tillage and enhanced aerobic decomposition, and by the structure of crop rotation, reduced input of plant residues or erosion losses. A change in soil acidity can testify to technological solutions (types of fertilisers, amelioration) as well as to differences in the natural soil background. For this reason, sets of indicators were regarded as essential for "isolating" the causal chain: physical indicators for relating changes to the level of mechanical disturbance, chemical ones for the input and mobility of substances, and biological ones for the reactivity of processes and functions. In such a combined approach, the resolution of the diagnosis was increased, and the likelihood of wrong causal conclusions was minimised.

It has also been shown that the variability in soil health status within agroecosystems was not only determined by the differences between farms, but also by the variability of practices within the same crop and even within the same region (Omer *et al.*, 2024). This has direct methodological consequences. First, the need for matching the scale of assessment with the real scale of heterogeneity of management increases, since agronomic solutions are often differentiated at the level of individual fields or even parts of fields. Second, when comparing locations, the history of management should also be taken into account, not just the current year, as some soil changes have a certain degree of inertia. Therefore, the evaluation of the soil status cannot be based on a single snapshot of indicators, without management context, since the same indicator value in different systems could have a different meaning (i.e. stabilisation, degradation, recovery from previous impacts).

A prerequisite for the correct interpretation of biological indicators is their dependence on soil context. It has been demonstrated that the use of soil classification improves the interpretation of biological soil health indicators and reduces the risk of false conclusions when comparing territories with different natural backgrounds (Congreves & Wu, 2024). This is because the basic soil properties determine the natural range for the variations in microbial and enzymic activities and faunal structure. Thus, direct comparison without stratification or normalisation may mask the management effect by mixing it with the inherent typological soil effect. In practical terms, this meant that the same tillage or fertilisation intensity could generate different amplitudes in the biological response, depending on texture, organic matter, carbonates, water regime, and thus a contextual interpretation was needed.

The comparison of the scientific results with the legislative framework of the European Union evidenced that moving toward systematic soil monitoring and evaluation of its resilience needed comparable indicators to capture the process of degradation and the changes in the stability over time (Directive (EU) 2025/2360, 2025). In this sense, sets of indicators acquired an additional value: they enabled the evaluations to be normalised, while keeping the sensitivity to local mechanisms of degradation. Thus, the soil indicators are viewed as the nucleus of the evaluation of anthropogenic impacts on agrocenoses, but their practical utility is enhanced only through the application of multicomponent approaches, synchronisation with the history of management and an explicit account for the soil-typological context.

Enzymes, microbial processes and soil fauna as risk indicators

It was evidenced that the biological indicators were more frequently used as an early warning tool to detect soil changes at an early stage, when the process of degradation was not always registered by conventional physico-chemical indicators. In the practice of assessing anthropogenic impacts, bioindication complements classical soil measurements, as it allows for the description not only of environmental conditions but also of the activity of soil processes associated with the transformation of organic matter and nutrients. In this sense, biological indicators are viewed as part of the "state" component within the "pressures – state – impacts" concept, as they directly reflect the response of the soil biota to the intensity of tillage, fertilisation or pollution.

Enzymatic assays were recognised as a widespread type of bioindication. It was stated that enzyme activity data supply valuable information on soil quality in agricultural soils and may also be employed for monitoring contaminated soils (Adetunji *et al.*, 2020; Lee *et al.*, 2020). The most popular enzymes were dehydrogenase, phosphatase, and urease because they are indicative of overall microbial metabolic activity, phosphorus transformation, and nitrogen cycling in soil. These tests are very handy as they are quantitative and can be compared between different managements or sites under varying pressure. Simultaneously, it was pointed out that the results of enzymatic bioindicators should be associated with soil conditions. Significant correlations were shown between pH of the soil solution and the activities of individual enzymes and between clay content and urease and phosphatase activities to illustrate the dependence of biological tests on native properties of soils (Lee *et al.*, 2020). A threshold concentration was also mentioned for residual mixed hydrocarbons in the soil at 1,000 mg/kg, above which the activity of individual enzymes can be shifted either to stimulation or inhibition (Lee *et al.*, 2020) (Table 1). In practical terms, such numerical benchmarks are used to justify that bioindicators should be analysed in conjunction with the fundamental properties of the soil and contamination data, rather than in isolation.

Table 1. Examples of numerical values for bioindicators and soil context

Indicator	Context	Value
Correlation pH – dehydrogenase (DH)	Relationship between soil properties and enzymatic activity	r = -0.704
Correlation pH – phosphatase (PHO)	Relationship between soil properties and enzymatic activity	r = -0.875
Correlation pH – urease (UR)	Relationship between soil properties and enzymatic activity	r = -0.784
Correlation clay – phosphatase (PHO)	Relationship between soil properties and enzymatic activity	r = 0.873
Correlation clay – urease (UR)	Relationship between soil properties and enzymatic activity	r = 0.930
Correlation of organic matter (OM) – phosphatase (PHO)	Relationship between soil properties and enzymatic activity	r = 0.698
Correlation dehydrogenase (DH) – phosphatase (PHO)	Relationship between enzymes	r = 0.804
Correlation phosphatase (PHO) – urease (UR)	Relationship between enzymes	r = 0.959
Threshold value for residual hydrocarbons in soil	Level at which enzyme activity changes were observed	1,000 mg/kg

Source: S.H. Lee *et al.* (2020)

Indicators of microbial processes and microbial biomass were described as tools reflecting differences between cropping systems and fertilisation regimes. In a case study performed on Indian vertisols, it was shown that enzymatic activity and microbial biomass characteristics can be employed as bioindicators of soil quality for comparison of different cultivation systems and fertilisation management practices (Ghosh *et al*., 2020). These arguments are in line with those where bioindicators are not only toxicant-sensitive but also reflect changes in the intensity of biogeochemical soil processes. Soil fauna, in particular earthworms, was mentioned in specialised reviews as a promising object for soils contaminated with pesticides. To sum up, in these studies, it was concluded that applying such approaches provided the possibility of using the population-level indicators, behavioural responses, and biomarkers to evaluate and characterise the state of soil under pesticide application, helping in monitoring activities (Duan *et al*., 2025). In Ukrainian agrocenoses, bioindication appeared to be an adequate tool for analysing the risks of using pesticides and detecting ecological risks caused by heavy metal pollution, thus confirming the applicability of biotic indices for agroecological diagnostics (Lishchuk *et al*., 2023a).

Generally, biotic indices appear to be best regarded as an early diagnosis, which complements the soil physico-chemical properties, thus increasing the sensitivity of agroecosystem state assessment to management variations. Their main practical value is seen in the possibility of comparing different technologies and fertilisation levels, as well as of evaluating the risks in the conditions of various types of chemical impact before irreversible changes are observed. At the same time, the correct interpretation of these indices requires taking into account the type of soil, the current season, and the observation scale to distinguish changes occurring under the natural variability from those caused by anthropogenic impact.

Diversity, dominance, and sensitivity indices in response to herbicide regimes and intensification

In a review of methods used to diagnose the state of the plant component of agrocenoses, it was concluded that the associated flora, mainly weeds, was most commonly used as an indicator of disturbance and selection pressure due to management. Across the disciplines, species richness and structure of plant communities, expressed by means of the Shannon diversity index and the Simpson dominance index, were the most frequently applied across crops and regions. Both indices were found to be useful in the context of quantifying the “simplification” of the plant communities due to intensive weed management and in detecting shifts toward communities with a limited number of taxa dominating cover and biomass. In the context of the assessment of human impacts, plants were most often used in the “state” part of the framework as they express the current structure of the phytocoenosis influenced by the repeated disturbances (tillage, herbicides, reduced crop rotation diversity) and as the “bridge” to the consequences such as the reduction of the floristic diversity, the loss of resource bases for other related trophic levels, and the reduction of the buffering capacity of field biota.

For cereals, it was revealed that the structure and diversity of weed communities were significantly different between crops and varied regionally, whereas diversity and dominance indices were considered suitable for the comparison between fields and regions. For example, in the cereal fields of South-Eastern Poland, the mean values of the Shannon diversity index of weed communities ranged from 2.11 to 2.52 (depending on the type of cereal), while the Simpson dominance index was relatively low and ranged between 0.08 and 0.18 (Table 2). This indicated a community structure without clear dominance of one species and the presence of differences between crop types, which confirmed the suitability of these indices for monitoring time and spatial changes (Sawicka *et al.*, 2020).

Table 2. Examples of plant indicators (indices, species richness, cover)

Object/context	Numerical indicators (examples)
Weeds in cereal crops, South-Eastern Poland	Shannon index (H): 2.11-2.52; Simpson dominance index: 0.080.18 (depending on crop)
Fallow land, 2014-2016, Northern China; annual applications of atrazine/tribenuron-methyl	Total species over 3 years: control 27; herbicide treatments 1923; number of species in control per year: 13 (2014), 14 (2015), 24 (2016)
Winter wheat, France; 1-20 years after conversion to conservation practices	Mean species richness: 23.9 species; mean Shannon index: 2.15
Forest-field ecotone, Poland; 1996-1998 vs. 2016-2018	Agrocenoses: 137 → 102 species; typical segetal species: 77 → 43; weed cover: ~32% → ~15%; QS (ecotone-agrocenosis): 0.47-0.64 → 0.30-0.55

Source: B. Sawicka *et al.* (2020), T. Skrajna (2020), Y. Qi *et al*. (2020), D. Derrouch *et al*. (2021)

The vulnerability of plant communities to herbicide applications was demonstrated mainly as a decrease in species richness and changes in the structure of plant communities. Changes occurred at both full and reduced exposure levels. This is important when evaluating risks to semi-natural components of the agricultural landscape and to the field boundaries, where the drift and run-off of active ingredients can be a chronically acting but "subtle" factor. According to the field experiment conducted by Y. Qi *et al*. (2020) on fallow plots in Northern China exposed to annual atrazine and tribenuron-methyl applications, the total number of species in the control was 27, and the number of species in the herbicide treatments ranged from 19 to 23, depending on the active ingredient and the dose. The authors also noted that the diversity indices were not always linearly related to the dose, and some effects were already observed at 25-50% of the recommended field concentration. Such observations (presented in this review) served as a methodological proof that the herbicide effect should not be considered as a separate factor, but as a regime, characterised by temporality, seasonality and transferability. In the "state" part, diversity indices should be accompanied by a species list and community structure.

For soil-conserving agriculture and reduced-tillage farming, it was found that the dynamics of floristic indicators can differ from those in intensive farming. After the introduction of conservation technologies, changes in the species composition of segetal communities and a trend towards homogenisation between fields were often registered, even against the background of a relatively stable mean diversity. In the network study of D. Derrouch *et al*. (2021), conducted on winter wheat in France (along the gradient of 1-20 years after the transition to conservation technologies), the average community was characterised by species richness of about 23.9 species and a Shannon index of about 2.15. This shows that it is not enough to judge intensification by a decrease in indices: some technological changes can lead to an increase in local species richness, and at the same time reduce the heterogeneity between fields, which in turn will change the ecological resilience of the agricultural landscape as a whole.

In the research of T. Skrajna (2020), which was aimed at long-term comparisons in the forest/woodland strip, ecotone, and field boundary system, it was found that intensification can lead to a direct impoverishment of the flora inside the agrocenosis, while the species pool in the ecotone has remained relatively preserved. In particular, in a 20-year comparison (1996-1998 and 2016-2018) in Poland, it was found that adjacent agrocenoses showed an approximately 30% decrease in the total number of species (from 137 to 102), a decrease in "characteristic" segetal species (from 77 to 43), and a decrease in the average weed coverage (from about 32% to 15%). At the same time, the ecotonal areas preserved a high taxonomic richness (about 171 and 169 taxa for the two periods), while there was a decrease in the floristic similarity between the ecotone and agrocenosis (the Sørensen coefficient QS decreased from 0.47-0.64 to 0.30-0.55) (Skrajna, 2020). This combination of evidence made it possible to infer a methodological implication that the context of the landscape and ecotonal diversity reservoirs should be taken into account when using plant indicators, as changes in the field may be obscured by the constancy of the surrounding semi-natural objects, but are associated with the increasing isolation of flora pools.

Geographic information systems, remote sensing, integral indices, and modelling as tools for scaling and scenario assessment

The collated evidence suggests that GIS and remote sensing (RS) of the Earth are not only used as additional "visualisation" tools, but as an independent level of analysis of the effects of anthropogenic impacts, which allow describing the state of agrocenosis in space and time, and extrapolate the results of local studies to the level of farms, river basins and regions. In practice, this became possible because the satellite time series contains repeated measurements over the growing season, while GIS provides the opportunity to combine the results of these measurements with the boundaries of fields, soil maps, management information and environmental context. Thus, RS is most often used to link point observations with generalisations at the level of the landscape, while GIS are used as a tool for integrating layers of data for spatial analysis and mapping risk zones.

In a review of phenological studies based on data from the Sentinel-2 satellite, G. Misra *et al*. (2020) found that the majority of studies employed traditional vegetation indices for characterising the phenological cycle. Red-edge and short-wave infrared (SWIR) bands were underutilised, although their inclusion can enhance the sensitivity to physiological conditions and stress of crops. In practical assessments of the impact of anthropogenic factors, this is important because simple indices are good at monitoring "greenness" and the overall productivity of plants, but are less able to identify the reasons for the changes. When the same decrease in an index could be caused by water stress, nutrient stress, herbicide stress or soil regime disturbance, the use of a wider spectral domain as well as phenological indicators (i.e. start, peak and end of the growing season, or integral of the growing season curve) is more robust for the interpretation of the "state" and for comparison between fields observed under different weather conditions.

The benefits of combining sensors for agromonitoring was further demonstrated in a multi-year phenology study, where the fusion of Sentinel-2 and Landsat 8 time series using the green Leaf Area Index (green LAI) in combination with Gaussian Processes Regression (GPR) increased the overall accuracy of growth period detection up to 74%, compared to 69% for Sentinel-2 and 63% for Landsat 8 (Amin *et al*., 2022). For the methodologies of anthropogenic impact, this was particularly valuable because, by stabilising the phenological signal, the fusion of satellite missions reduced time series gaps and decreased the dependence of assessments on isolated events of cloud presence or missing data. As a result, the comparability of satellite-based indicators across seasons, farming practices or areas, within a single growing season, was improved.

The operational integration of satellite-based products in agromonitoring systems of policy monitoring was exemplified in the Sen4CAP approach (2017), which underlined the importance of harmonised production pipelines to convert satellite data into reproducible indicators for monitoring farming practices at the field level. This was a relevant example of the transition of RS from imagery to infrastructure, and the shift from maps to production quality control and harmonised procedures. For integrated "pressure" indicators, the application of the Harmonised Risk Indicator 1 (HRI 1) was standardised, where the 2011 to 2013 time slice was considered as a temporal reference for comparison, and the "use and risk" components were aggregated according to the classification of active substances (Eurostat, n.d.). This was useful since it allowed for the standardised comparison between the dynamics of pesticide pressure between different years and facilitated the inclusion of other layers in GIS analysis. For agrocenoses assessment, it facilitated the integration of pressure (e.g. regional risk profile of applications) with spatial "state" indicators (phenological anomalies, field heterogeneity) and with impact models.

In the sources reviewed, modelling was mainly seen as a tool for scenario-based analysis, as a way to build on observations, and to move beyond the description of a "state" to the prediction of potential "impacts". In the case of an integrated approach to modelling sustainable intensification, there was an emphasis on the usefulness of combining model predictions with monitoring and satellite data for decision support and scenario analysis of changes in practices (Brown *et al*., 2023). Studies related to the soil, land use, and climate interactions pointed out that the stability of the agroecosystem depends on the holistic consideration of those, since the human impact is coming from the interaction of the management and climate drivers, which are often confounded (Si *et al*., 2025). Therefore, GIS and RS provided spatial extrapolation and pattern recognition, integrated indices provided a standardised way of representing "pressures", and models allowed for scenario-based extrapolation of likely outcomes under different management regimes.

The comparison of the selected sources revealed that the highest level of agreement between studies occurred when anthropogenic pressure was conceptualised as a bundle of factors, rather than a single overriding pressure, and when the conditions for comparability (such as scale, natural baseline, management history) were clearly outlined. The highest levels of disagreement occurred when the same indicators were applied as generic proxies, without specific relation to soil, landscape or geochemical conditions, which necessarily changed the meaning of "state", even when the absolute values were the same. In the systematic review protocol by S.M. Beyene *et al*. (2025) stressed that several anthropogenic drivers can change both the direction and strength of plant and soil feedbacks; the agroecosystem is not expected to respond as a fixed function of a single driver. This claim was consistent with other research where agroecosystem status was assessed based on functional traits, but it was inconsistent with other research where indices were used outside of a covariate context, since in the latter case, the relationships between drivers are masked, leading to contradictory results among studies.

In the review on bioindicators by B. Gorain & S. Paul (2021), the authors showed that biotic indicators responded to changes in management sooner than less reactive physical-chemical attributes, and were useful for early warning signs of degradation. This was consistent with risk assessment methods based on sensitive bioindicators, but it was not entirely consistent with methods where limits are transposed between soils and seasons, since the authors concluded that soil type and season significantly affected the baseline variability of bioindicators. In the chapter on earthworms as bioindicators, V. Dhiman & D. Pant (2022) demonstrated that biomarkers and behavioural responses at the sub-individual level may respond faster to toxic effects than population-level responses, and may aid in early risk detection. This reasoning supported the idea of early bioindicators for soil quality, but it was not consistent with methods where bioresponses are interpreted in the absence of information on contaminants and soil, since in this case, the same biomarker may respond to different mechanisms of stress and may lead to contradictory results among studies.

In the study by J. Špulerová *et al*. (2022) on natural and semi-natural features as indicators of biocultural value, it was demonstrated that the features are not accidental but are structurally constitutive elements, which facilitate connectivity and the capacity to maintain biodiversity. This was consistent with perspectives that relate the stability of agroecosystems to environmental diversity and structure, but it was not consistent with evaluations carried out exclusively at the field scale and in the absence of a landscape context, since the same pressures in different contexts may have different ecological consequences. In the research on spatio-temporal dynamics of rural areas, D. Statuto *et al*. (2019) showed that using spatial metrics and multi-temporal map comparison enables quantifying the trend and speed of land cover changes; detecting areas where changes occurred at different intensities, and thus with a different capacity for sustainable planning. This is in line with a landscape-oriented approach, where the spatial structure of the mosaic is viewed as a contextual factor modifying the response to a treatment rather than as an independent factor with its own effect. Simultaneously, the authors stressed that temporal context is necessary for the interpretation of landscape metrics and that information on land use history is needed because the metrics express the footprint of past land uses, and when ignoring land use history, wrong conclusions could be drawn about the causes of land cover change. In the study on agro-biodiversity and agro-ecosystem stability, S. Wang (2021) found that agro-biodiversity within agro-ecosystems contributes to system resilience to disturbance and fluctuations, and thus should be considered a functional attribute of resilience, rather than a structural attribute. This is in line with the landscape-oriented approach, which stresses the importance of environmental mosaics; it is less in line with assessment approaches based on single productivity metrics because productivity and stability may not always be synchronised, especially when multiple drivers are involved.

In the comparative study of arsenic, T. Tarvainen *et al*. (2020) found that high concentrations of arsenic in agro-ecosystems may be due to elevated geochemical background or anthropogenic impact, and that the same concentrations may have a different environmental meaning depending on the source and geochemical context. This supports the need for contextual interpretation of the bioindicator, but does not support the widespread practice in applied studies where high concentration values are associated with high levels of anthropogenic impact without separating geochemical background concentrations, which leads to incomparability of results between regions and to the risk of misattribution. In this context, the study of P. Rendon *et al*. (2020) is of interest, which investigated the relationship between the state of an agroecosystem and the service of regulation of soil erosion. In this study, the indicator assessment was closely linked to the functional consequences with practical implications for risk management. This study has some similarities with the spatial approach, since erosion is a process that has marked spatial heterogeneity. Based on the results of all the studies, it was possible to identify which blocks of results were most similar and which were less comparable. The greatest comparability was found for the principle of multicausality and contextuality, as it was supported by both a mechanistic model of multiple drivers and geochemical comparisons that distinguish between background levels and anthropogenic load. In contrast, attempts to extrapolate absolute thresholds and scales of biological responses to different soil types and management conditions without standardising the context were less comparable, since some studies considered the context as a primary modifier, while in other studies it was implicit. In general, the most consistent explanation of the variability of the results was obtained by a comprehensive logic in which the structure of the landscape defined the framework of vulnerability, the bioindicators captured the early sensitive changes in state, the geochemical context prevented false attributions, and functional indicators such as the regulation of erosion allowed the understanding of the consequences in terms of ecosystem services.

CONCLUSIONS

A methodological review of the assessment of the anthropogenic impact on agrocenoses indicated that the most reproducible results were obtained when pressure indicators, state indicators, and consequence indicators were integrated into a cause-and-effect model suitable for territorial and temporal comparisons. It was verified that agrocenoses should be conceived as hierarchical systems, in which technological management regimes modify soil processes, the structure of the associated vegetation, and the functioning of the biota, while ecological responses are often non-linear and contextual. Under these conditions, indicator approaches based on a single block of indicators were insufficient, while a complete set of physical, chemical, and biological parameters of the soil provided a more consistent interpretation of the changes and prevented false attributions of causality.

It was corroborated that biological indicators -microbial processes, enzyme activity, and responses of soil fauna- constituted sensitive markers of the early changes in state and risk of degradation or pollution, and in addition, improved the functional interpretation of the results. In the plant compartment, diversity and dominance indices and functional indicatorscaptured the selective pressure of intensification and herbicide management, and were informative for the diagnosis of simplification of plant communities. Indicators of landscape heterogeneity calculated with the geographic information systems provided a complementary vision that considered environmental mosaics as modifiers of vulnerability and resilience. Remote sensing and integral indices were conceived as scaling tools that allowed articulating field data with spatial patterns and supporting the scenario-based justification of management practices. Future studies should be directed to standardising protocols for comparing indicators between different soil types and climates, validating integral indices against time series of long-term monitoring, and deepening the integration of remote sensing, biological, and modelling information to assess non-linear changes and predict management responses.

ACKNOWLEDGEMENTS

None.

FUNDING

None.

CONFLICT OF INTEREST

None.

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Методичні підходи до оцінювання антропогенного впливу на агроценози

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Анотація. Актуальність дослідження зумовлена посиленнями антропогенного впливу на агроценози через інтенсифікацію землеробства, хімічні навантаження та просторові трансформації землекористування, що одночасно змінює ґрунтові процеси, структуру супутньої рослинності й функціонування біоти. Мета роботи полягала в систематизації та зіставленні сучасних методичних підходів до оцінювання антропогенного навантаження на агроценози в логіці «тиски – стан – наслідки». Виконано тематичний синтез публікацій 2019-2025 років із відбором джерел, що містили чіткі індикатори та правила їх обчислення; узагальнення здійснено за блоками ґрунт – біота – рослинність – геопросторові інструменти. Показано, що найбільш відтворювані оцінки забезпечувало комбінування фізичних, хімічних і біологічних показників ґрунту, оскільки одновимірні метрики втрачали чутливість до управлінських тисків і не відображали системних змін. Встановлено, що біоіндикація на основі ферментативної активності, мікробних процесів і реакцій ґрунтової фауни була придатною для раннього виявлення деградаційних зрушень і токсикологічних ризиків до появи стійких змін у фізико-хімічних властивостях. Підтверджено, що рослинні індекси різноманіття та домінування разом із функціональними індикаторами фіксували селективний тиск інтенсифікації та гербіцидних режимів, відображаючи спрощення угруповань і зміщення видового складу. Обґрунтовано, що геоінформаційні системи та дистанційне зондування Землі підвищували порівнюваність оцінок через просторово-часове масштабування, виявлення внутрішньопольової неоднорідності й картування зон ризику. Визначено, що інтегральні індекси підсилювали зіставність між територіями, але потребували узгодження напрямів інтерпретації показників, прозорого зважування та врахування невизначеності. Висновки підтвердили, що інтегрований підхід, що поєднував ґрунтові, біотичні, рослинні та геопросторові індикатори, був найбільш придатним для моніторингу, порівняння територій і підтримки управлінських рішень щодо мінімізації негативного впливу агровиробництва на довкілля

Ключові слова: інтенсифікація; здоров'я ґрунту; біоіндикація; ландшафтна гетерогенність; дистанційне зондування

Received: 03.10.2025 Revised: 20.01.2026 Accepted: 26.02.2026 Published: 07.04.2026

BIOLOGICAL SYSTEMS: Theory and Innovation

ISSN 2706-8382
e-ISSN 2706-8390

Journal homepage: https://bioscience.com.ua/en
Vol. 17, No. 1, 2026, 52-66

UDC 632.5:632.934/935

DOI: 10.31548/biologiya/1.2026.52

The effectiveness of applying products with herbicidal active substances in soybean crops

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Abstract. The aim of the study was to determine the level of biological effectiveness of soybean protection systems and to rationally combine the mechanisms of action of herbicidal formulations with the use of the adjuvant Skaba EC. The effectiveness of herbicide active substances on weed infestation and soybean yield was evaluated. The index of competitive pressure of weeds on crops was determined. Under all studied conditions, a clear hierarchy of effectiveness between two- and three-component systems was observed. Differences between application rates were much less pronounced than expected. When using 100% herbicide rates in combination with the adjuvant Skaba EC (0.2 L/ha) in two-component systems, the average reduction in weed numbers was: for annual broadleaf weeds – 80.0-91.4%, for annual grasses – 72.0-92.0%, and for perennial broadleaf weeds – 69.0-81.0%. In the three-component system, these values were 95.1%, 94.0%, and 87.0%, respectively. Reducing the herbicide rate to 75% combined with an increased adjuvant rate (0.3 L/ha) did not result in a significant decrease in weed control effectiveness. In the three-component system, the values reached 93.9%, 94.5%, and 85.0%, respectively. A 25% reduction in herbicide rates, when combined with the adjuvant, had little effect on the final biological outcome, while significantly reducing the chemical load on the agroecosystem. The combination of inhibitors provided the highest biological effectiveness of weed control. The level of weed infestation in soybean crops during the years of study depended significantly on the composition of the herbicide protection system and the combination of products with different mechanisms of action of active substances on plants. Two-component systems without the use of an adjuvant ensured moderate weed reduction (58-88%), whereas three-component multisite combinations increased control effectiveness to 85-97% for annual species and 76-88% for perennial species (and under conditions with an adjuvant – up to 90-97% and 84-88%, respectively). When applying optimised (reduced by 3-40%) herbicide rates in combination with the maximum adjuvant rate (0.4 L/ha), weed control efficiency remained consistently high

Keywords: adjuvant; agricultural technologies; weeds; competitive pressure index; efficacy index

Suggested Citation:
Sykalo, O., & Konopolskyi, O. (2026). The effectiveness of applying products with herbicidal active substances in soybean crops. *Biological Systems: Theory and Innovation*, 17(1), 52-66. doi: 10.31548/biologiya/1.2026.52.

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INTRODUCTION

Soybean is one of the key strategic crops in modern crop production in Ukraine due to its high content of complete plant protein and oil, as well as its ability for symbiotic nitrogen fixation. It plays an important role in the formation of sustainable agroecosystems, which defines it as one of the significant crops for agricultural production. This is evidenced by data on the soybean market in Ukraine, which shows considerable fluctuations. According to the State Statistics Service of Ukraine (n.d.), in 2024 the cultivation area reached up to 2.63 million hectares, in 2025 – up to 2.42 million hectares, while in 2026 it was expected that the total area would remain at about 2 million hectares. Successful soybean cultivation requires consideration of both the biological characteristics of the crop – its requirements for heat, moisture and light intensity – and specific agronomic practices. However, one of the main limiting factors in realising soybean yield potential remains a high level of weed infestation, caused both by the biological traits of the crop (slow initial growth and weak competitiveness during the first 30-40 days of vegetation) and by the diversity of weed species in the Forest-Steppe zone. According to M.I. Kyrychok (2023), weeds emerging within 3 to 21 days after soybean emergence are capable of creating significant competition with crop. Untimely weed control in soybean crops, or its absence, results in yield losses of 15-30%. Under mixed weed infestation, yield losses may reach 30-50% or more, while seed quality declines, accompanied by deterioration of sowing and market characteristics, as noted by M. Kyrychok & S. Remeniuk (2022). Thus, the chemical method of weed control remains a fundamental component of modern soybean cultivation technologies worldwide, particularly under intensive production systems and high competitive pressure from weeds within the agrophytocenosis.

At the same time, the combination of available active substances with favourable soil and climatic conditions creates numerous opportunities for experimental research. A. Dykun *et al*. (2020) reported that the application of a two-component post-emergence herbicide containing bentazone and imazamox significantly reduced weed density in soybean crops under mixed infestation due to its high biological activity. Double application of the herbicide in combination with the adjuvant Metolat, with a two-week interval, achieved effectiveness at the level of 89% reduction of *Setaria glauca* (Poir.) and 65% of *Echinochloa crus-galli* (L.), while control of broadleaf weeds reached 99%, with a 97% reduction in fresh weed biomass. M.I. Kyrychok (2023) also emphasised that when using herbicides with a pronounced soil effect in soybean crops, a single application is advisable. Thus, the application of the herbicide Pari, SL (active substance imazethapyr, 100 g/L) at a rate of 1.0 L/ha resulted in the destruction of 89.0% of weed seedlings, whereas split application (0.3 L/ha followed by 0.4 L/ha after four days) resulted in 78.3% weed control. A similar pattern was observed with the herbicide Fabian, WG (imazethapyr 450 g/kg + chlorimuron-ethyl 150 g/kg), where the full rate provided 83.8% effectiveness, while split application (0.03 kg/ha followed by 0.04 kg/ha after four days) reduced effectiveness to 72.6%. Studies on the effectiveness and phytotoxicity of herbicides in various combinations in soybean crops were also conducted by M. Meseldžija *et al*. (2020) in Serbia. It was found that the most effective treatments were combinations of metribuzin + S-metolachlor + imazamox + oxasulfuron + thifensulfuron-methyl, with overall effectiveness ranging from 96.98% to 97.40%. Acceptable phytotoxicity levels in soybean over two years of study were observed when applying herbicides combining bentazone + imazamox + thifensulfuron-methyl.

New technologies for the simultaneous detection of weeds and targeted herbicide spraying in soybean crops have been investigated by researchers in the United States, including T.H. Avent *et al*. (2024). However, their studies were primarily related to application techniques involving products with the active ingredient dicamba and the post-emergence use of glufosinate, which ultimately resulted in a reduction in herbicide application rates. Based on these findings, they reduced the amount of herbicide applied while maintaining weed control at a level of 93%. The reduction of weed impact on soybean plants was studied by F.C. Cardoso *et al*. (2024) in Pakistan using the active ingredient flumioxazin. The effectiveness of post-emergence herbicides in soybean crops in Thailand was investigated by U.R.R. Pamungkas *et al*. (2025), whose experimental schemes included herbicides containing three active ingredients: fluazifop-P-butyl, fomesafen and clethodim. The effects of herbicides with different active ingredients on weed control efficiency and crop response were examined by M. Younesabadi *et al.* (2024) and S. Hamza *et al.* (2025), who studied the action of thifensulfuron-methyl. In turn, F.C. Cardoso *et al.* (2024) analysed the role of adjuvants in enhancing the effectiveness of herbicide treatments. At the early stages of organogenesis, soybean is characterised by slow growth and development, which results in increased sensitivity to competition from weeds for light, moisture and mineral nutrients. Under such conditions, even short-term deterioration of the phytosanitary status of crops may lead to a significant reduction in photosynthetic activity, limited leaf area formation and yield losses. In agricultural practice, increasing the proportion of soybean in crop rotations may have considerable consequences. Associated weed species – annual grasses and broadleaved weeds – serve both as reservoirs of diseases and pests and as a factor contributing to increased pesticide pressure on the agroecosystem and additional yield losses (Ivashchenko & Ivashchenko, 2019).

This problem can be prevented if an effective herbicide protection scheme is in place, as repeatedly highlighted in the works of A. Dykun *et al.* (2020), M. Kyrychok *et al.* (2022) and F.C. Cardoso *et al.* (2024). Modern herbicides are generally characterised by narrow biological selectivity, which reduces their effectiveness under conditions of a multi-component weed community and necessitates the use of tank mixtures and higher application rates. This, in turn, increases pesticide load on agroecosystems, raises production costs and enhances environmental risks. Contemporary systems of chemical protection of soybean crops are based on the use of herbicides with different modes of action, allowing targeted control of major biological groups of weeds and minimising the risk of resistant biotypes formation. Of particular importance in this context is the combination of products that inhibit different metabolic pathways in weed plants, including photosynthetic processes, amino acid synthesis and lipid biosynthesis in cell membranes (Kyrychok, 2023). The biological effectiveness of different soybean herbicide protection systems is determined by their influence on competitive interactions within the agrophytocoenosis and their selectivity towards specific weed groups. In order to enhance the realisation of herbicidal effects and enable a reduction in application rates without loss of biological and economic efficiency, particular attention was given to the role of the adjuvant or surfactant in two- and three-component systems and their multisite mechanisms of action. The results of the aforementioned studies underline the relevance of selecting appropriate herbicide treatments to optimise weed control and increase soybean productivity under varying seasonal conditions. The aim of this study was to evaluate the biological effectiveness of different soybean crop protection systems and to substantiate the rational combination of herbicides with different mechanisms of action in conjunction with the use of the adjuvant Skaba EC.

MATERIALS AND METHODS

Field experiments to determine the effectiveness of herbicide application and the impact of tank mixtures of herbicides on weed infestation in soybean were conducted at the Separated Subdivision of the National University of Life and Environmental Sciences of Ukraine "Agronomic Research Station" (NULES of Ukraine ARS), located in the village of Pshenychne, Vasylkiv district, Kyiv region, during 2023-2025. The soil of the experimental plots at NULES of Ukraine ARS is classified as typical low-humus chernozem, coarse silty, medium loam, with a humus content of 4.5%, a salt extract pH of 6.8-7.0, a cation exchange capacity of 31.9 mg-eq per 100 g of soil, and a clay particle content of 20-25% according to granulometric composition. The soil type is typical for this zone. The arable layer (0-30 cm) has a granular-dusty structure, while the sub-arable layer has a nutty-granular structure. The groundwater level lies at a depth of 2-4 m. The parent material, carbonate loess, occurs at a depth of 180-210 cm and contains 9-11% calcium carbonates. Chernozem soils are characterised by high natural fertility and a significant content of total and available forms of nutrients. In particular, the 0-20 cm soil layer contains 0.27-0.31% total nitrogen, 0.15-0.25% total phosphorus, and 2.3-2.5% potassium. The content of available phosphorus (according to Machigin) is 3.3-3.4 mg, and available potassium is 9.8-10.3 mg per 100 g of soil. The physicochemical and water-physical properties of the soil of the experimental field are presented in Tables 1 and 2.

Table 1. Physicochemical characteristics of the soil of the experimental field of NULES of Ukraine ARS (2023-2025)

Sampling depth, cm	Humus content, %	Salt extract pH	Carbonate content, %	Cation exchange capacity, mg-eq per 100 g of soil
0-10	4.53	6.87	–	31.9
35-45	4.38	7.30	1.66	32.0
45-55	1.36	7.30	9.20	19.1
130-140	0.86	7.30	10.50	15.0
210-230	–	7.30	9.70	–

Source: compiled by the authors

Table 2. Water-physical properties of typical low-humus chernozem at NULES of Ukraine ARS (2023-2025)

Horizon depth, cm	Bulk density, g/cm³	Total porosity, %	Maximum water-holding capacity, %	Wilting moisture, % of MWHC	Capillary rupture moisture, %	Field water capacity, %	Total water capacity, %
5-25	1.25	52	13.6	10.8	19.7	28.2	41.6
25-45	1.16	55	13.2	10.7	19.1	27.3	47.4
80-100	1.27	52	12.3	9.8	17.9	25.6	41.0
135-155	1.20	54	–	m	15.0	21.5	45.0
155-185	1.20	56	12.0	9.6	14.6	20.8	48.3
230-250	1.55	42	–	–	15.4	22.1	27.1

Notes: MWHC – maximum water-holding capacity; m – missing value
Source: compiled by the authors

Tables 1 and 2 demonstrate that the upper soil horizons contain the highest levels of humus and are characterised by a neutral pH with high cation exchange capacity, whereas the deeper layers are distinguished by an increased carbonate content and reduced water-physical properties. Total porosity and water-retention capacity decrease with depth, which limits water availability for roots in deeper layers and determines optimal conditions for crop development within the upper horizons. Thus, soil characteristics create favourable conditions for shallow root development and influence the effectiveness of herbicide application and agronomic practices. Weather conditions during the 2023 growing season were characterised by a contrasting combination of factors: an excessively wet April contributed to the accumulation of soil moisture reserves, while extremely dry conditions in May and August limited the early stages of plant growth and the grain-filling process, respectively. Among the years studied, 2024 was the most stressful during the generative development phases due to an abnormally dry and hot July (10 mm of precipitation; average temperature 23.5°C), which directly explains the potential reduction in the realisation of yield structure components. In 2025, weather conditions were characterised by relative stability following a "warm + precipitation deficit" pattern from May onwards, particularly in July-August, resulting in water availability consistently acting as a limiting factor throughout key ontogenetic stages (leaf area formation → pod setting → grain filling). Field experiments to determine the effectiveness of herbicide active ingredients on weed infestation, their application efficiency, and soybean yield were conducted according to the methodologies of V. Moysyeyenko & V. Yeshchenko (1994), S.O. Trybel *et al*. (2001), and M.I. Kyrychok (2023).

The following herbicides were used in the experimental studies:

1. Basagran, SL (active ingredient: bentazone) belongs to photosystem II inhibitors (Herbicide Resistance Action Committee (HRAC) group C3). Its mode of action is associated with blocking electron transport in the light phase of photosynthesis,

leading to rapid suppression of photochemical processes in susceptible weeds. The product exhibits predominantly contact action and is most effective against annual broadleaved weeds at the 2-4 leaf stage. Limited systemic activity results in reduced effectiveness against perennial species, which is confirmed by the obtained data on weed density and dry biomass. Application rate: 1.5-2.0 L/ha, single application.

2. Harmony 75 WG (thifensulfuron-methyl) belongs to acetolactate synthase (ALS) inhibitors and is classified in HRAC group B. Its mechanism of action involves blocking the synthesis of essential amino acids, resulting in the cessation of weed growth processes. The product is characterised by high selectivity towards soybean and effectiveness against a wide range of broadleaved weeds, including some perennial species, when applied at the 2-6 leaf stage. Application rate: 0.025-0.035 kg/ha, single application.

3. Pulsar, SL (imazethapyr) also belongs to ALS inhibitors but differs in its systemic activity and ability to be absorbed through both leaf surfaces and the root system. This determines its effectiveness not only against broadleaved weeds but also against certain grass species, as well as its prolonged control of emerging weeds during the early stages of crop development. Maximum effectiveness is observed from the emergence stage to the 4-leaf stage of weeds, which is consistent with the experimental results obtained. Application rate: 0.5-1.0 L/ha, single application.

4. Select, EC (clethodim) is a representative of acetyl-CoA carboxylase (ACCase) inhibitors, HRAC group A. Its mechanism of action is associated with disruption of lipid synthesis in cell membranes, leading to the death of grass weeds. The product is effective against both annual and perennial grass species at the 2-6 leaf stage and is an essential component of soybean protection systems against grass weed infestations. Application rate: 0.14-0.28 L/ha, single application.

5. The surfactant Skaba EC does not possess its own herbicidal activity but plays an important role in enhancing the effectiveness of herbicides. Its use improves leaf surface wetting, increases the penetration of active ingredients into weed tissues, and enhances their translocation. This creates conditions for reducing herbicide application rates without loss of biological effectiveness, which is clearly reflected in the indicators of weed dry biomass, competitive pressure index, and herbicide efficiency index.

The analysis included annual grasses and broadleaf weeds, as well as perennial species, in particular common pigweed (*Amaranthus retroflexus* L.), common thistle (*Cirsium arvense* (L.) Scop.), lamb's quarters (*Chenopodium album* L.), barnyard grass (*Echinochloa crus-galli* L.), common fumitory (*Fumaria officinalis* L.), cleavers (*Galium aparine* L.), common bindweed (*Polygonum convolvulus* L.), common knotweed (*Polygonum persicaria* L.), grey foxtail (*Setaria glauca* L.), field mustard (*Sinapis arvensis* L.), black nightshade (*Solanum nigrum* L.), yellow sow thistle (*Sonchus arvensis* L.), field pennycress (*Thlaspi arvense* L.) and other species. The total area of the plots in the herbicide trials was 32 m^2, and that of the survey plots was 25 m^2, with three replicates and a randomised arrangement of treatments. The weed species composition was determined by transect surveys and weed identification within the survey plots (divided into annual grasses, annual dicots and perennial species). Weed abundance (plants/m^2) was determined using the frame method on permanent survey plots:

- the survey area was defined by a frame of fixed size (typically 0.25 m^2 or 0.5 m^2);
- surveys were conducted in several replicates on each plot;
- surveys were conducted prior to the application of herbicides (background) and at specified intervals after treatment (28 days), as well as before harvest (according to the experimental design).

The dry weight of weeds (g/m^2) was determined by sampling weed biomass from the survey area, followed by drying in an oven to constant weight (60-65°C) and conversion to 1 m^2. The reduction in weed abundance/dry weight, %, was calculated relative to the control:

$$E\ (\%) = \frac{C - T}{C} \times 100, \qquad (1)$$

where C – control group score, T – test group score.

The Weed Competitive Pressure Index (WCPI) was defined as an integrated measure of the competitive impact of the weed component on the soybean agro-phytocenosis (based on the relative values of weed abundance/dry weight compared to the control or the minimally weeded treatment –

in accordance with the algorithm adopted in this study). The Herbicide Efficacy Index (HEI) was calculated as a generalised indicator of the protection system's effectiveness, reflecting the relationship between the level of weed suppression and the crop response (yield/productivity), which allows for the comparison of full, reduced and optimised herbicide rates and the role of surfactants.

The methodology for calculating the herbicide efficacy index (HEI) was based on determining the reduction in dry weed mass compared to the control without herbicide application, where the dry weed mass was 120 g/m²:

$$\text{HEI} = \frac{\text{Reduction in dry weed mass,\%}}{\text{Herbicide rate index}}, \qquad (2)$$

where the rate index is: 100% rate without surfactant → 1.00; 100% rate + surfactant → 1.00; 75% rate + surfactant → 0.75; optimised rate + surfactant (-33…40%) → 0.67.

The higher the HEI value, the more efficiently a unit of herbicide load is utilised. Experimental data were processed using methods of variation statistics: mean values and variability indices were calculated; the significance of differences between treatments was assessed (analysis of variance, significance criteria at $p \leq 0.05$); and relationships between variables were determined using correlation analysis (Pearson's coefficient). During the study, only herbicides included in the State Register of Pesticides and Agrochemicals Allowed for Use in Ukraine were used, in compliance with the standards set by the Food and Agriculture Organization of the United Nations (n.d.).

RESULTS AND DISCUSSION

Table 3 presents the characteristics of the studied herbicides in terms of active substance, classification according to the Herbicide Resistance Action Committee/Weed Science Society of America (HRAC/WSSA), mechanism of action, spectrum of controlled weeds, and the stage of maximum application effectiveness. This approach allows for substantiating the selection of product combinations in protection systems and provides a more detailed explanation of the experimental results obtained.

Table 3. Mechanism of action and biological target of the studied active substances

Content of active substance in the formulation	Product	HRAC/ WSSA group	Mechanism of action	Main weed group	Stage of maximum effectiveness
Bentazone, 480 g/L	Basagran, SL	C3 (6)	Photosystem II inhibitor (blocks electron transport)	Annual broadleaf weeds	2-4 leaf stage
Thifensulfuron-methyl, 750 g/kg	Harmony, WG	B (2)	ALS inhibitor (disrupts amino acid synthesis)	Broadleaf weeds, including some perennials	2-6 leaf stage
Imazethapyr, 100 g/L	Pulsar, SL	B (2)	ALS inhibitor (systemic action)	Broadleaf weeds + some grasses	emergence – 4 leaf stage
Clethodim, 120 g/L	Select, EC	A (1)	ACCase inhibitor (disrupts lipid synthesis)	Annual and perennial grasses	2-6 leaf stage
–	Adjuvant Skaba EC	–	Enhances wetting, penetration, and translocation	–	applied with herbicides

Notes: ALS – acetolactate synthase; HRAC – Herbicide Resistance Action Committee; WSSA – Weed Science Society of America

Source: compiled by the authors

Herbicides with the active ingredient bentazon belong to photosystem II inhibitors (HRAC group C3). Their action is associated with blocking electron transport in the light phase of photosynthesis, which leads to rapid suppression of photochemical processes in susceptible weeds. These products act mainly by contact and are most effective against annual broadleaf weeds at the 2-4 leaf stage. Limited systemic activity results in reduced effectiveness against perennial species, which is confirmed by the obtained indicators of weed density and dry biomass. Herbicides containing the active ingredient thifensulfuron-methyl belong to ALS inhibitors and are classified in HRAC group B. Their mode of action involves blocking the synthesis of essential amino acids, leading to the cessation of growth processes in weeds. These products are characterised by high selectivity towards soybean and effectiveness against a broad spectrum of broadleaf weeds, including some perennial species, when applied at the 2-6 leaf stage. Products containing the active ingredient imazethapyr also belong to ALS inhibitors; however, they differ in their systemic action and ability to be absorbed both through the leaf surface and the root system. This determines their effectiveness not only against broadleaf weeds but also against some grass weeds, as well as prolonged control of weed emergence in the early stages of crop development. Maximum effectiveness of these products is observed from emergence to the 4-leaf stage of weeds, which is consistent with the experimental data obtained. Herbicides containing the active ingredient clethodim are representatives of acetyl-CoA carboxylase (ACCase) inhibitors, HRAC group A. Their mode of action is associated with disruption of lipid synthesis in cell membranes, leading to the death of grass weeds.

These products are effective against annual and perennial grass species at the 2-6 leaf stage and are an essential component of soybean protection systems against grass weed vegetation. The surfactant Skaba EC has no inherent herbicidal activity; however, it plays an important role in enhancing the effectiveness of the products. Its use improves wetting of the leaf surface, increases the penetration of active substances into weed tissues and enhances their translocation. This creates conditions for reducing herbicide application rates without loss of biological effectiveness, which is clearly reflected in the indicators of weed dry biomass, the competitive pressure index and the herbicide efficiency index. The use of a surfactant in multi-component systems improved adhesion of the working solution, accelerated penetration of active substances into weed tissues and enhanced their translocation. This created conditions for reducing herbicide rates without loss of biological effectiveness, as confirmed by high HEI values and reduced WCPI in the respective experimental variants. Thus, Table 3 serves as a methodological basis for further analysis of the research results and allows explanation of the advantages of multisite soybean protection systems, in which photosystem II, ALS and ACCase inhibitors are combined to ensure maximum biological effect and stable weed control. Table 4 summarises the results of evaluating the effectiveness of the studied soybean herbicide protection systems in terms of combining mechanisms of action of active substances at specific product concentrations, their biological impact on the weed component of the agrophytocenosis and the practical consequences for crop productivity formation.

Table 4. Biological justification of the effectiveness of protection systems considering the mechanism of action of active substances

Protection system with products containing active substances	Combination of mechanisms of action	Biological effect	Practical outcome
Bentazone, 480 g/L + clethodim, 120 g/L	PSII + ACCase	Control of broadleaf weeds + grasses	Limited effectiveness against ALS-sensitive species
Thifensulfuron-methyl, 750 g/kg + clethodim, 120 g/L	ALS + ACCase	Strong control of broadleaf weeds and grasses	High selectivity, but risk of ALS resistance
Imazethapyr, 100 g/L + clethodim, 120 g/L	ALS (systemic) + ACCase	Broad spectrum of action, soil and foliar activity	Stable weed control in crops
Bentazone, 480 g/L + thifensulfuron-methyl, 750 g/kg + clethodim, 120 g/L	PSII + ALS + ACCase	Multisite action	Maximum effectiveness and resistance minimisation
All treatments + adjuvant	Enhanced penetration	Faster action and reduced crop stress	Improved photosynthetic potential

Source: compiled by the authors

Crop protection system based on the combination of products with active ingredients (bentazon, 480 g/L + clethodim, 120 g/L) – a photosystem II (PSII) inhibitor and an ACCase inhibitor – provided effective control of annual broadleaf and grass weeds at early stages of their development. The biological effect of this combination was realised through disruption of photosynthetic processes in broadleaf species and inhibition of lipid synthesis in grass weeds. At the same time, the limited systemic action resulted in reduced effectiveness against perennial weeds, which was reflected in relatively higher values of residual dry biomass and the competitive pressure index. The combination of products with active ingredients (thifensulfuron-methyl, 750 g/kg + clethodim, 120 g/L), containing an ALS inhibitor and an ACCase inhibitor, was characterised by high biological activity against broadleaf weeds due to the suppression of essential amino acid synthesis, while also ensuring reliable control of grass species. However, the highly specific mode of action of the ALS inhibitor created a potential risk of resistance development under prolonged use of this system without rotation of active substances, which justifies its combination with products from other classes. The application of a system based on a systemic ALS inhibitor in combination with an ACCase inhibitor (imazethapyr, 100 g/L + clethodim, 120 g/L) provided the most balanced weed control due to the combination of soil and foliar activity. Such a system effectively suppressed both annual and certain perennial weed species, resulting in a stable reduction in their density and dry biomass throughout the growing season. The biological action of this combination was reflected in reduced competitive pressure on the crop and the creation of favourable conditions for soybean growth and development. The highest biological and economic efficiency was achieved with three-component protection systems combining PSII, ALS and ACCase inhibitors (bentazon, 480 g/L + thifensulfuron-methyl, 750 g/kg + clethodim, 120 g/L). The multisite mode of action of this combination of active substances allowed simultaneous impact on different metabolic pathways in weed plants, ensuring maximum reduction in both the density and dry biomass of annual and perennial weeds. These systems were characterised by the lowest values of the weed competitive pressure index and the highest values of the herbicide efficiency index, which is consistent with the results of the correlation analysis. The results of weed density assessment indicate a significant differentiation in the effectiveness of soybean protection systems depending on the combination of herbicide active substances, their mechanisms of action and the use of a surfactant. On average over the years of study, weed density reduction ranged from 58% to 96%, indicating substantial differences in the biological effectiveness of the tested variants. Under the use of a two-component system with active ingredients bentazon, 480 g/L + clethodim, 120 g/L, the reduction in annual broadleaf weeds averaged 71.3%, annual grasses – 79.0%, whereas effectiveness against perennial broadleaf weeds was significantly lower at 59.0% (Table 5). This indicates the limited action of the contact photosystem II inhibitor and insufficient systemic activity against perennial species.

Table 5. Reduction in weed numbers in treatments without surfactant (100% rate), %

Weed species	Bentazone, 480 g/L + + clethodim, 120 g/L	Thifensulfuron-methyl, 750 g/kg + + clethodim, 120 g/L	Imazethapyr, 100 g/L + + clethodim, 120 g/L	Bentazone, 480 g/L + + thifensulfuron-methyl, 750 g/kg + + clethodim, 120 g/L
Amaranthus retroflexus L.	74	88	80	91
Cirsium arvense (L.) Scop.	58	70	66	76
Chenopodium album L.	72	85	82	90
Echinochloa crus-galli L.	78	65	84	88
Fumaria officinalis L.	69	84	77	87
Galium aparine L.	65	80	75	85
Polygonum convolvulus L.	70	83	78	88
Polygonum persicaria L.	71	82	79	89
Setaria glauca L.	80	68	86	90
Sinapis arvensis L.	73	87	80	91
Solanum nigrum L.	72	85	79	89
Sonchus arvensis L.	60	72	68	78
Thlaspi arvense L.	76	86	81	92
Other species	70	80	75	85

Source: compiled by the authors

Replacing a product containing the active ingredient bentazone, 480 g/L, with a product containing the ALS inhibitor active ingredient thifensulfuron-methyl, 750 g/kg (the thifensulfuron-methyl, 750 g/kg + clethodim, 120 g/L) ensured improved control of broadleaf weeds: the reduction in the abundance of annual broadleaf species increased to 84.4%, and that of perennial species to 71.0%, which is 10-12% higher than in the variant using products containing the active ingredient bentazone, 480 g/L. At the same time, control of grass weeds was less consistent at 66.5% (65-68% by species), which is explained by the lack of soil activity of the ALS inhibitor against these species. The combination of formulations containing the active ingredients imazethapyr, 100 g/L + clethodim, 120 g/L was characterised by a more balanced effect. The reduction in the abundance of annual broadleaf weeds reached 79.0%, perennial broadleaf weeds – 67.0%, and annual grass weeds – 85.0%. Compared with the formulation containing the active ingredients thifensulfuron-methyl, 750 g/kg + clethodim, 120 g/L, efficacy against perennial broadleaf weeds was 6% higher, due to the systemic action of imazethapyr. The highest efficacy among the variants without surfactants was provided by the three-component system of bentazone, 480 g/L + thifensulfuron-methyl, 750 g/kg + clethodim, 120 g/L, where the reduction in the abundance of annual broadleaf and grass weeds reached 89.1% and 89.0%, respectively, and that of perennial broadleaf weeds – 77.0%. Thus, compared with two-component regimens, the efficacy of perennial weed control increased by 14-18%, confirming the advantage of a multi-site mode of action. The addition of an adjuvant (surfactant) significantly enhanced the biological efficacy of all control systems. Thus, in the bentazone, 480 g/L + clethodim, 120 g/L + surfactant treatment, the reduction in the abundance of annual broadleaf weeds increased from 71.3% to 80%, and that of perennial weeds from 59.0% to 69.0%, i.e. by 10%. A similar trend was observed for grass weeds, where efficacy increased by 8% (Table 6).

Table 6. Weed density in treatments with 100% rate + adjuvant Skaba EC (0.2 L/ha), %

Weed species	Bentazone, 480 g/L + + clethodim, 120 g/L	Thifensulfuron-methyl, 750 g/kg + + clethodim, 120 g/L	Imazethapyr, 100 g/L + + clethodim, 120 g/L	Bentazone, 480 g/L + + thifensulfuron-methyl, 750 g/kg + + clethodim, 120 g/L
Amaranthus retroflexus L.	82	94	87	97
Cirsium arvense (L.) Scop.	68	80	76	86
Chenopodium album L.	80	92	89	96
Echinochloa crus-galli L.	86	74	91	95
Galium aparine L.	74	88	82	92
Fumaria officinalis L.	78	91	84	94
Polygonum convolvulus L.	79	90	85	94
Polygonum persicaria L.	80	89	86	95
Setaria glauca L.	88	76	93	96
Sinapis arvensis L.	82	94	87	97
Solanum nigrum L.	81	92	86	95
Sonchus arvensis L.	70	82	78	88
Thlaspi arvense L.	84	93	88	97
Other species	78	88	83	92

Source: compiled by the authors

In the system thifensulfuron-methyl, 750 g/kg + clethodim, 120 g/L + surfactant, the reduction in annual broadleaf weed density reached 91.4%, and perennial species – 81.0%, which is 7.0% and 10.0% higher than without the surfactant. Control of grass weeds also increased; however, it remained somewhat lower compared with systems containing the active ingredient imazethapyr. The system imazethapyr, 100 g/L + clethodim, 120 g/L + surfactant provided a consistently high level of control of all weed groups: 86-92% for annuals and 77% for perennial species. Compared with the corresponding variant without a surfactant, effectiveness increased on average by 7-9%. The highest values were recorded in the three-component system bentazon, 480 g/L + thifensulfuron-methyl, 750 g/kg + clethodim, 120 g/L + surfactant, where the reduction in annual broadleaf weeds reached 95.1%, and perennial weeds – 87.0%. This is 6-10% higher than in the three-component variant without a surfactant and 15-20% higher than in two-component systems. Data in Table 7 indicate that the use of a reduced (75%) rate of herbicides in combination with the adjuvant Skaba EC (0.3 L/ha) provided a high level of weed control in soybean crops, which in most cases was not inferior to, and in some cases even approached, the efficacy of the full rate of the products without a surfactant. In the variant bentazon, 480 g/L + clethodim, 120 g/L (75% rate + surfactant), the reduction in annual broadleaf weeds averaged 78.0%, annual grasses – 73.0%, while effectiveness against perennial broadleaf weeds was lower at 67.0%. Compared with the corresponding variant without a surfactant, control effectiveness increased by 6-8%, indicating improved realisation of the contact action of bentazon due to enhanced penetration of the product into weed tissues. In the system thifensulfuron-methyl, 750 g/kg + clethodim, 120 g/L (75% rate + surfactant), the level of reduction in broadleaf weeds was significantly higher. Thus, annual broadleaf species were suppressed by 89.4%, and perennial species by 79.0%. At the same time, control of grass weeds remained somewhat lower at 73% for annual species, reflecting the specificity of the ALS inhibitor's mode of action.

Table 7. Weed abundance under treatments with 75% herbicide rate + the adjuvant Skaba EC (0.3 L/ha), %

Weed species	Bentazone, 480 g/L + clethodim, 120 g/L	Thifensulfuron-methyl, 750 g/kg + clethodim, 120 g/L	Imazethapyr, 100 g/L + clethodim, 120 g/L	Bentazone, 480 g/L+ + thifensulfuron-methyl, 750 g/kg + + clethodim, 120 g/L
Amaranthus retroflexus L.	80	92	85	96
Cirsium arvense (L.) Scop.	66	78	74	84
Chenopodium album L.	78	90	87	95
Echinochloa crus-galli L.	84	72	90	94
Galium aparine L.	72	86	80	90
Fumaria officinalis L.	76	89	82	93
Polygonum convolvulus L.	77	88	83	92
Polygonum persicaria L.	78	87	84	93
Setaria glauca L.	85	74	92	95
Sinapis arvensis L.	80	92	85	96
Solanum nigrum L.	79	90	84	94
Sonchus arvensis L.	68	80	76	86
Thlaspi arvense L.	82	91	86	96
Other species	76	86	81	90

Source: compiled by the authors

The application of the imazethapyr, 100 g/L + clethodim, 120 g/L (75% rate + surfactant) treatment provided the most balanced weed control among the two-component treatments. The reduction in the number of annual broadleaf weeds reached 84%, perennial broadleaf weeds – 75%, and annual grasses – 91%. Compared with the thifensulfuron-methyl system, 750 g/kg + clethodim, 120 g/L, the efficacy of control of perennial broadleaf weeds was 5% higher, and for perennial grasses by 8%, which is due to the systemic action of imazethapyr and the partial soil activity of the formulation. The highest reductions in weed populations were recorded in a three-component formulation containing the active ingredients bentazone, 480 g/L + thifensulfuron-methyl, 750 g/kg + clethodim, 120 g/L (75% rate + surfactant). In this variant, the abundance of annual dicotyledonous weeds decreased by an average of 93.9%, perennial broadleaf weeds by 85%, and annual grasses by 94.5%. Compared with two-component systems, the efficacy of perennial weed control was 16-18% higher, indicating the decisive role of the multi-site mode of action. The results presented in Table 8 indicate that the use of optimised (reduced by 33-40%) herbicide rates in combination with an increased rate of the surfactant Skaba EC (0.4 L/ha) ensured a consistently high level of weed control, comparable to that of full-rate variants, and in some cases practically identical. The overall level of weed reduction under this treatment ranged from 67% to 97%, depending on the composition of the protection system and the biological group of weeds.

Table 8. Weed abundance under treatments with optimised herbicide rates + the adjuvant Skaba EC (0.4 L/ha), %

Weed species	Bentazone, 480 g/L + + clethodim, 120 g/L	Thifensulfuron-methyl, 750 g/kg + + clethodim, 120 g/L	Imazethapyr, 100 g/L + clethodim, 120 g/L	Bentazone, 480 g/L + + thifensulfuron-methyl, 750 g/kg + + clethodim, 120 g/L
Amaranthus retroflexus L.	81	93	86	97
Cirsium arvense (L.) Scop.	67	79	75	85
Chenopodium album L.	79	91	88	96
Echinochloa crus-galli L.	85	73	91	95
Fumaria officinalis L.	77	90	83	94
Galium aparine L.	73	87	81	91
Polygonum convolvulus L.	78	89	84	93
Polygonum persicaria L.	79	88	85	94
Setaria glauca L.	86	75	93	96
Sinapis arvensis L.	81	93	86	97
Solanum nigrum L.	80	91	85	95
Sonchus arvensis L.	69	81	77	87
Thlaspi arvense L.	83	92	87	97
Other species	77	87	82	91

Source: compiled by the authors

In the variant bentazon, 480 g/L + clethodim, 120 g/L (optimised rate + surfactant), the reduction in annual broadleaf weeds averaged 79.0%, perennial broadleaf weeds – 68.0%, and annual grasses – 85.5%. Compared with the corresponding 75% rate variant, the effectiveness of control was practically the same, with differences not exceeding 1-2%, indicating sufficient realisation of herbicidal action even with further reduction in the rate of active substances. In the system thifensulfuron-methyl, 750 g/kg + clethodim, 120 g/L (optimised rate + surfactant), control of broadleaf weeds remained high: the density of annual broadleaf weeds decreased by 90.4% and perennial species by 74.0%, which is 10-12% higher than in the variant with bentazon. Control of grass weeds was somewhat lower at 80.0% for annual species, which is consistent with the mechanism of action of the ALS inhibitor and the absence of soil activity against grass weeds. The use of the combination of active ingredients imazethapyr, 100 g/L + clethodim, 120 g/L (optimised rate + surfactant) provided one of the best balances between effectiveness and reduced chemical load. This is particularly relevant in soybean cultivation and reduces the risk of herbicide injury to plants. The reduction in annual broadleaf weeds was 85.0%, perennial broadleaf weeds – 76.0%, and annual grasses – 92.0%. Compared with the combination thifensulfuron-methyl, 750 g/kg + clethodim, 120 g/L, the effectiveness of control of perennial broadleaf weeds was higher by 5% and perennial grasses by 8%, which is due to the systemic action of imazethapyr and its prolonged effect. The maximum reductions in weed density under the optimised rate were recorded in the three-component system bentazon, 480 g/L + thifensulfuron-methyl, 750 g/kg + clethodim, 120 g/L (optimised rate + surfactant). In this variant, the density of annual broadleaf weeds decreased on average by 94.9%, perennial broadleaf weeds by 86.0%, and annual grasses by 95.5%. Compared with two-component systems, the effectiveness of perennial weed control was higher by 16-18%, while compared with the corresponding 75% rate variants, the differences did not exceed 1-2%, confirming the high stability of the multisite mode of action.

Numerous scientific studies demonstrate the high biological efficacy of herbicide formulations containing a combination of the active ingredients bentazone, thifensulfuron-methyl, clethodim and imazamox in soyabean crops. They provide reliable weed control, contribute to increased crop productivity and, when used in accordance with application guidelines, do not exhibit phytotoxic effects on soybean plants. According to S.Z. Knezevic *et al*. (2019), the most effective and, at the same time, critical period for applying herbicides against dicotyledonous weeds is the 1-3 true trifoliate leaf stage of soybean, whereas treatments against grass weeds can be carried out regardless of the crop's developmental stage, but, as a rule, before the onset of flowering. The present study confirmed the findings of P. Grassini *et al*. (2015) that, in order to reduce the costs of chemical weed control and minimise the environmental impact of herbicides, post-emergence herbicides in soybean crops should be applied in combination with adjuvants, which enhances the biological efficacy of the active ingredients and allows for the optimisation of their application rates. The first post-emergence herbicide introduced for use against dicotyledonous weeds was Bazagran, 48% w/w (active ingredient – bentazone). The specific effects of the product in soyabean crops were observed by K.S. Gill & M.A. Arshad (1995) and P.H. Graham & C.P. Vance (2003). In soybean crops, the herbicide successfully controlled common chickweed, spreading bittercress, buckwheat, field mustard, wild radish, common ragwort, field thistle, black nightshade, common ragwort and others. When applied at the 1-3 true leaf stage at a rate of 2-3 L/ha, weed infestation in soyabean crops was reduced by 76-82%, and weed biomass was reduced by 87-97%. Evidently, the high efficacy of the herbicide was observed in the absence of grass weeds that exhibit resistance to Basagran. The data obtained confirmed the results of a study by V.M. Zherebko & O.V. Dykun (2022), which showed that the application of the herbicide Pas, 10% w/v, to emerging crops at rates of 0.05-0.1 L/ha of active ingredient reduced weed biomass by 74-91%, whilst yield increased by 2.8-3.0 times. An important advantage of this herbicide is its broader spectrum of action on weed species. O. Hurmanchuk *et al*. (2021) found that in some years the herbicide exhibits negative after-effects, causing stunting, plant death and thinning in the crops of subsequent rotation. Although the use of herbicides does not usually cause direct yield losses and is effective against weeds, any stressful effect may adversely affect the growth and development of soya plants. Stress factors that alter the physiological state of the crop can disrupt seed formation and development processes and reduce seed viability. Applying herbicides at suboptimal times or failing to follow application guidelines may result in chemical stress and plant burns, which in some cases leads to a significant reduction in yield or even crop failure, as noted by M. Knežević *et al.* (2008) and Y. Kawasaki *et al.* (2016). The aforementioned studies have shown that the application of post-emergence herbicides in combination with adjuvants ensures a high level of weed control, optimises the application rates of active ingredients and increases soybean yield; however, the efficacy and safety of the product depend to a large extent on adherence to the recommended application stages and guidelines.

CONCLUSIONS

Comparative analysis showed that the effectiveness of soybean weed control systems was determined not so much by the absolute rate of herbicides as by the rational combination of modes of action of active substances and the use of the adjuvant (surfactant) Skaba EC. In all studied treatments, a clear hierarchy between two- and three-component systems was observed; however, differences between application rates were less pronounced than expected. When applying 100% herbicide rates with the adjuvant in two-component systems, the average reduction in weed numbers was 80-91% for annual broadleaf weeds, 72-92% for annual grasses, and 69-81% for perennial broadleaf weeds. In the three-component system (bentazone + thifensulfuron-methyl + clethodim + adjuvant), the respective values reached 95.1%, 94.0%, and 87.0%. Reducing herbicide rates to 75% with an increased adjuvant rate (0.3 L/ha) had little effect on weed control efficiency, providing 78-89%, 73-91%, and 67-79% for two-component systems and 93.9%, 94.5%, and 85% for the three-component system, respectively. Optimised reduced herbicide rates (33-40%) combined with the maximum adjuvant rate (0.4 L/ha) also ensured consistently high control: 79-90%, 74-92%, and 68-80% in two-component systems and 94.9%, 95.5%, and 86% in the three-component system. The difference compared with the 100% rate + adjuvant did not exceed 1-3%, remaining within the limits of experimental error. The level of weed infestation largely depended on the composition of the herbicide protection system and the combination of products with different modes of action. Two-component systems without an adjuvant provided moderate weed reduction (58-88%), whereas three-component multisite combinations increased effectiveness to 85-97% for annual species and 76-88% for perennial species; with the adjuvant, these values increased to 90-97% and 84-88%, respectively. The highest biological efficacy was achieved through the combination of photosystem II, ALS, and ACCase inhibitors. The three-component system of bentazone + thifensulfuron-methyl + clethodim showed the lowest values of weed dry mass (42-50 g/m^2 with adjuvant), which was 16.7-41.7% lower than in two-component systems. The use of the adjuvant additionally reduced weed dry mass by 26-30 g/m^2 and increased control effectiveness by 6.8-10% compared with treatments without the adjuvant. Thus, increasing the adjuvant rate compensated for the reduction in herbicide rates, ensuring full realisation of the mechanisms of action of active substances and reducing the chemical load on the agroecosystem. Future research should focus on the systematic study of the effectiveness of integrated use of reduced herbicide rates in combination with various surfactants under different soil and climatic conditions, with the aim of optimising weed control and minimising chemical load on the agroecosystem.

ACKNOWLEDGEMENTS

None.

FUNDING

None.

CONFLICT OF INTEREST

None.

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Ефективність застосування препаратів з гербіцидними діючими речовинами на посівах сої

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Анотація. Метою дослідження було визначити рівень біологічної ефективності систем захисту сої та раціонально поєднати механізм дії препаратів з гербіцидними властивостями та застосуванням ад'юванта Скаба КЕ. Визначено ефективність діючих речовин гербіцидів на забур'яненість і врожайність посівів сої. Визначали індекс конкурентного тиску бур'янів на посіви. За всіх досліджуваних фонів спостерігалася чітка ієрархія ефективності між дво- та трикомпонентними системами. Відмінності між нормами внесення препаратів були значно менш вираженими, ніж очікувалося. За використання 100 % норми гербіцидів у поєднанні з ад'ювантом Скаба КЕ (0,2 л/га) у двокомпонентних системах середні значення зниження чисельності бур'янів становили: для однорічних дводольних – 80,0-91,4 %, для однорічних злакових – 72,0-92,0 %, для багаторічних дводольних – 69,0-81,0 %. У трикомпонентній системі поєднання препаратів ці показники становили відповідно 95,1 %, 94,0 % і 87,0 %. Перехід до 75 % норми гербіцидів у поєднанні з підвищеною нормою ад'юванту (0,3 л/га) не призводив до істотного зниження ефективності контролю бур'янів. У трикомпонентній системі значення досягали 93,9 %, 94,5 % і 85,0 % відповідно. Зменшення норми гербіцидів на 25 % за умови використання ад'юванту практично не впливало на кінцевий біологічний ефект, але водночас суттєво знижувало хімічне навантаження на агроекосистему. Поєднання інгібіторів забезпечувало найвищу біологічну ефективність контролю бур'янів. Рівень забур'яненості посівів сої у роки досліджень істотно залежав від складу системи гербіцидного захисту та поєднання препаратів із різними механізмами впливу на рослину діючих речовин. Двокомпонентні системи без використання ад'юванту забезпечували помірне зниження чисельності бур'янів (58-88 %), тоді як трикомпонентні мультисайтові комбінації підвищували ефективність контролю до 85-97 % для однорічних і 76-88 % для багаторічних видів (а на фонах із ад'ювантом – до 90-97 % та 84-88 % відповідно). За застосування оптимізованих (знижених на 3-40 %) норм гербіцидів у поєднанні з максимальною нормою ад'юванту (0,4 л/га) ефективність контролю бур'янів залишалася стабільно високою

Ключові слова: ад'ювант; агротехнології; бур'яни; індекс конкурентного тиску; індекс ефективності

Received: 30.09.2025 Revised: 26.01.2026 Accepted: 26.02.2026 Published: 07.04.2026

BIOLOGICAL SYSTEMS: Theory and Innovation

ISSN 2706-8382
e-ISSN 2706-8390

Journal homepage: https://bioscience.com.ua/en
Vol. 17, No. 1, 2026, 67-77

UDC 577.27:576.36

DOI: 10.31548/biologiya/1.2026.67

Macrophage signalling pathways and their regulation by cellular microenvironment factors

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Abstract. Macrophages are key cells of innate immunity that play an important role in regulating the inflammatory response, tissue repair processes, and the development of various pathological conditions, in particular, cancer and chronic inflammatory diseases. The functional activity of macrophages is largely determined by microenvironment signals, including cytokines, hormones, growth factors, and pathogen-associated molecular structures. The purpose of the study was to summarise contemporary scientific data on the molecular mechanisms of regulation of functional activity of macrophages under the influence of microenvironment factors and analyse the interaction of signalling pathways that determine their polarisation and functional plasticity. The paper analysed the results of contemporary studies on the regulation of macrophage signalling cascades involving Th1- and Th2-type cytokines, glucocorticoids, transforming growth factor β (TGF-β), and signalling pathways activated by pathogen-associated molecular structures. Activation of receptor complexes, in particular TLR4, has been shown to trigger complex signalling cascades associated with activation of the transcription factor NF-κB and MyD88-dependent signal transmission mechanisms. It was found that the interaction of various signalling molecules can lead to both synergistic and antagonistic effects affecting the expression of genes associated with the inflammatory response, cell adhesion, and lipid metabolism. The generalised data obtained confirmed the concept of a continuum of functional states of macrophages, according to which these cells are able to change their phenotype depending on the conditions of the microenvironment. These results highlighted the importance of investigating the mechanisms of interaction between different microenvironment signals to understand the regulation of macrophage functions. The practical significance of the study lies in the systematisation of recent ideas about the mechanisms of macrophage regulation, which can be used for further research on the molecular basis of the immune response, and for the development of new approaches to the treatment of inflammatory, autoimmune and oncological diseases

Keywords: immune response; TGF-β; cytokines; tumour; growth factors

INTRODUCTION

The cytokine environment of the tumour microenvironment and the nature of the inflammatory infiltrate of tumours often show signs of a chronic inflammatory process that combines the characteristics of both Th1- and Th2-mediated immune responses. Such a mixed immune profile plays an important role in regulating the functional activity of innate immune cells, in

Suggested Citation:
Andriushchenko, A. (2026). Macrophage signalling pathways and their regulation by cellular microenvironment factors. *Biological Systems: Theory and Innovation*, 17(1), 67-77. doi: 10.31548/biologiya/1.2026.67.

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particular macrophages, which are key components of the tumour microenvironment. Research by X. Chen (2022) showed that macrophages can acquire various functional phenotypes under the influence of cytokines, growth factors, and other signalling molecules present in the tissue environment. This phenotypic plasticity determines their involvement in the development of inflammatory responses, tissue remodelling processes, and tumour progression. One of the important signalling factors involved in the development of the tumour microenvironment is transforming growth factor β (TGF-β), which can modulate the functional activity of various cells of the immune system. In particular, it has been shown that TGF-β can significantly affect macrophage differentiation and polarisation, contributing to the development of phenotypes associated with anti-inflammatory activity and tissue remodelling. In turn, X. Bai *et al*. (2025) described the role of inflammatory signalling cascades in regulating cellular response. In addition, macrophage activation can occur under the influence of tissue damage signals that trigger specific molecular mechanisms of cell polarisation during sterile inflammation (Koncz *et al*., 2023). According to C. Chen *et al*. (2023), epigenetic regulatory mechanisms that are activated by microenvironment signalling molecules can control the expression of genes associated with macrophage polarisation, thereby determining their functional state in the processes of inflammation and tissue regeneration. Recently, the role of cytokine receptor-related signalling pathways in the development of specific macrophage phenotypes has attracted particular attention. Increased expression of receptors for individual cytokines can significantly alter the functional properties of these cells. In the context of the tumour microenvironment, it is important to increase the expression of the IL-17BR receptor, which, according to current research, may have prognostic significance in some types of malignancies, in particular in breast cancer. X. Gao *et al*. (2023) showed that activation of appropriate signalling pathways can alter immune responses in tumour tissue and affect the interaction between tumour cells and immune system cells. Given that macrophages can express various receptors for cytokines and growth factors, it can be assumed that the increased expression of IL-17BR observed in breast tumour tissues may be associated with stimulation of macrophages by signalling molecules of the tumour microenvironment, in particular TGF-β. The study by J. Song *et al*. (2024) showed that signalling pathways activated by this factor can alter the transcription programmes of cells and promote the development of functional phenotypes similar to tumour-associated macrophages. Such cells are characterised by the ability to support chronic inflammation, stimulate angiogenesis, and modulate the immune response in the tumour microenvironment.

Interest in studying the effect of TGF-β on macrophages is also related to historical understanding of the sensitivity of these cells to this factor. For a long time, it was believed that mature macrophages are practically unable to respond to TGF-β. K. Zhang & C. Jagannath (2025) showed that monocytes can express a significant number of receptors for this cytokine and respond to its stimulation by reducing inflammatory activity. Simultaneously, the expression of the corresponding receptors decreases during differentiation into macrophages, which leads to a decrease in the sensitivity of cells to TGF-β. However, recent studies show that the response of macrophages to TGF-β can largely depend on the conditions of their differentiation and the composition of the cellular microenvironment. For example, it has been shown that a combination of different signalling stimuli can alter the expression of receptors and the activity of the corresponding signalling pathways. The study by J. Zhang *et al*. (2025) demonstrated that post-transcriptional regulatory mechanisms, in particular m6A-type RNA modifications, can influence the expression of genes associated with macrophage polarisation and promote the development of alternatively activated cell phenotypes. In addition, under physiological conditions, the processes of differentiation and functional activation of macrophages can be influenced by hormonal factors, in particular glucocorticoids. The natural glucocorticoid cortisol is constantly present in the mammalian circulation, which can modulate immune responses. A synthetic analogue of cortisol is dexamethasone, which is widely used in experimental studies to model the effect of glucocorticoids on immune system cells. C. Chen *et al*. (2025) found that glucocorticoids can affect signalling pathways that control the functional state of macrophages, in particular, by altering cellular protein localisation and regulating intercellular communication. Thus, current data have shown that the development of a functional macrophage phenotype is determined by the complex interaction of signalling molecules of the tumour microenvironment, epigenetic mechanisms, and metabolic processes. Understanding these mechanisms is important for explaining the role of macrophages in the development of the tumour process and may contribute to the development of new approaches to cancer therapy. The purpose of the study was to systematise and critically analyse current ideas about the molecular mechanisms of regulation of functional activity of macrophages in the context of the influence of microenvironment factors, and to elucidate the role of interaction of signalling pathways in the processes of their polarisation and functional plasticity.

MATERIALS AND METHODS

The study was of a review and analytical nature and aimed at systematising current scientific data on the molecular mechanisms of regulation of macrophage signalling pathways under the influence of cellular microenvironment factors. The object of the study was macrophage signalling pathways activated by cytokines, growth factors, hormones, and pathogen-associated molecular structures. The study focused on the molecular mechanisms regulating macrophage polarisation, their functional plasticity, and the interactions among key signalling cascades that determine the development of distinct functional phenotypes of these cells in inflammatory and tumourigenic processes. The search for scientific sources was carried out in the international bibliographic databases PubMed, Scopus, Web of Science, and Google Scholar. The following keywords were used to identify relevant publications: macrophage polarisation, macrophage signalling pathways, tumour microenvironment, cytokine signalling, NF-κB signalling, and TGF-β signalling. The analysis mainly included publications published in 2020-2025 that contain experimental or review data on the mechanisms of macrophage activation. The selection criteria were scientific originality of the results, indexing of the publication in international scientometric databases, and compliance with the research topic.

Contemporary studies on the regulation of macrophage functional activity and polarisation were used to establish the theoretical foundation of the research. As part of the study, the approaches proposed by T. Gauthier *et al*. (2023) and A.S.M. Moin *et al*. (2021) were applied to analyse the mechanisms of macrophage activation through M1/M2 polarisation and their functional diversity was evaluated depending on the microenvironment. Special attention was paid to the plasticity of macrophages and their ability to adapt the functional phenotype in response to cellular environmental conditions. The analysis also considered the concept that macrophages can form different phenotypes by responding to signals coming from the microenvironment. The interpretation of the obtained data was carried out based on the concept of functional diversity of macrophages and the integration of signalling pathways that ensured the adaptation of cells to changes in the tissue environment. The study of macrophages of atherosclerotic plaque and their effect on lipid metabolism and inflammatory processes was carried out based on the studies by J.O. Abaricia *et al*. (2020) and E.M. O'Brien & K.L. Spiller (2022). Systematisation of macrophage activation mechanisms under the influence of growth factors and cytokines was carried out in accordance with the approach by Y.H. Lin *et al*. (2020). A significant aspect was the processes of cellular interaction and regulation of the immune response of macrophages. The paper used the method of systematic analysis of literature, which provided for the selection, classification, and critical evaluation of scientific publications. To identify general patterns in the regulation of macrophage signalling pathways, the method of comparative analysis was used, enabling a comparison of the results of various experimental studies. In addition, the method of synthesis of scientific data was applied, which corresponded to the approaches of meta-analytical generalisation of findings and allowed integrating information from various scientific sources.

RESULTS AND DISCUSSION

Identification of the conditions necessary to preserve the ability of macrophages to respond to TGF-β allowed investigating the effect of this growth factor on gene expression in macrophages. An increase in the expression of a number of transcription-regulating factors involved in the regulation of the immune response and cell adhesion was observed. In addition, genes that are responsible for lipid metabolism can be classified into a separate group. The identified genes are characteristic of macrophages involved in chronic inflammatory processes, such as, for example, atherosclerosis. The correspondence of the resulting macrophage phenotype to macrophages found in atherosclerotic plaque can be traced primarily by the expression of genes involved in lipoprotein metabolism. Thus, J.O. Abaricia *et al.* (2020), E.M. O'Brien & K.L. Spiller (2022) and H. Lin *et al.* (2025) showed that atherosclerotic plaque macrophages express apolipoprotein E (APOE), lectin-like receptor for oxLDL-1 (LOX or OLR1), apolipoprotein C-II (APOC2), sortilin-like receptor L (SORL1 or LR11), LRPAP1, and ABC transporter ABCG1. All of these genes are involved in the regulation of blood cholesterol and are somehow associated with the pathogenesis of atherosclerosis (Boutilier & Elsawa, 2021).

However, macrophages of atherosclerotic plaque, which have a phenotype characteristic of the chronic inflammatory process, are similar in many respects to tumour-associated macrophages. The most striking example of this correspondence is the increased expression of enzymes involved in the synthesis of leukotriene B4. Thus, oesophageal tumour samples showed increased expression of ALOX5AP and LTA4 hydrolase, enzymes necessary for the synthesis of leukotriene B4, whose role in tumour progression and metastasis has been shown to be extremely important, in particular for renal cell carcinoma (Peng *et al.*, 2020). Although the phenotype of macrophages obtained in culture when stimulated with IL-4, dexamethasone, and TGF-β reflects only some of the properties of tumour-associated macrophages, it can be argued that the resulting model reflects some of the properties necessary for these cells to participate in tumour pathogenesis. To approach the physiological situation, it is necessary to include in the model other cytokines produced by the tumour, signals received by macrophages as a result of intercellular contacts, and physical factors such as hypoxia. The complexity of the experimental system indicates the feasibility of using mathematical and analytical approaches to more effectively solve the corresponding problems. The analysed data confirmed that macrophages in the human body cannot be divided into only two categories – classically and alternatively activated (Eming *et al.*, 2021). The system of mononuclear phagocytes can be visualised as a continuum of functional states, that is, polarisation: at one of the poles of which there are macrophages that actively restore tissue after suppressing the inflammatory response, and at the other – macrophages that actively stimulate inflammation (Fig. 1).

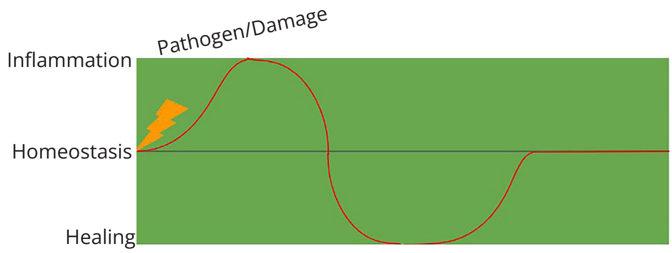

Figure 1. Changes in the macrophage phenotype during the inflammatory response process
Source: compiled by the author

Figure 1 illustrates the transformation of the macrophage phenotype in response to damage or pathogens, showing the transition from a state of homeostasis to inflammatory activation and subsequent tissue healing. A neutral state corresponding to healthy, undamaged tissue can be located between these two poles. The diversity of macrophage phenotypes is explained not only by the different combinations of factors affecting the cell, but also by the sequence of influence of these factors. Macrophages treated with interferons (IFN) a few hours before LPS stimulation were able to produce large amounts of tumour necrosis factor (TNF), in addition, IFN treatment leads to a change in the response to interleukin-10 (IL-10) (Scott *et al.*, 2023; He *et al.*, 2024). The ability of macrophages to respond to various stimuli regardless of the phenotype and degree of differentiation led to the creation of the concept of macrophage phenotype plasticity (Zhu *et al.*, 2022). According to this concept, macrophages are considered as cells that have the ability to change their state within a continuum of possible states depending on changes that have occurred in their environment. Regardless of the phenotype, macrophages can respond adequately to pathogen-associated molecules (PAMPs), such as LPS or muramyl dipeptide (MDP). In addition, macrophages involved in stimulating the inflammatory response can respond to anti-inflammatory signals and reduce their inflammatory potential (Strizova *et al.*, 2023). Cells that have similar adaptation potentials and means to stimulate the strongest inflammatory responses or fibrotic processes pose a potential danger to the body. Over the course of evolution, macrophages have demonstrated the ability to perform their functions smoothly. Their ability to accurately determine the nature and degree of exposure required by the body in each specific situation is the result of complex mechanisms of regulation and adaptation. Macrophages have a receptor system of the highest complexity, which allows them to take an active part in almost any physiological process. Macrophages express receptors for Th1 and Th2 cytokines, hormones, and chemokines, which allows them to integrate innate and acquired immunity. Toll-like receptors and other molecular structure recognition receptors allow macrophages to detect most types of pathogens with high efficiency. A wide range of scavenger receptors, Fc receptors, and complement component receptors make macrophages universal cleaners (Oliver *et al.*, 2024). This complexity of the receptor system guarantees the detection of all signals necessary for an effective and timely immune response without exception. However, a highly developed receptor system alone is not enough to form the correct response. The specificity of the macrophage response is determined by complex signal transmission pathways within the cell, which are activated after binding of the ligand to its receptor. For many receptors, there is evidence that the signalling pathway activated by them is not a unidirectional chain of events. Typically, receptor-activated signalling cascades are branched structures that include positive and negative feedback loops that affect the signal chain at different levels (Sim *et al.*, 2022).

An example of a branched signal transmission pathway is a signalling pathway that is activated by LPS and leads to TNF production. The first control node of this reaction is the stage of recognition of LPS by the receptor complex. To be recognised, LPS must be bound to a lipopolysaccharide-binding protein (LBP). This complex, in turn, binds to TLR4 via the CD14 coreceptor and MD2 adapter protein (Xia *et al.*, 2023). The absence of any component of this system leads to a significant decrease in the cell's sensitivity to LPS. Furthermore, this system allows adjusting the cell to a certain level of sensitivity. Thus, the presence of membrane-bound CD14 on the cell surface is required to identify low LPS concentrations, while the presence of soluble CD14 is sufficient to identify high LPS concentrations (Li *et al.*, 2020). After recognition by the LPS receptor complex, the adapter protein MyD88 binds to TLR4, which, in turn, leads to activation of IκB kinase (IKK) and phosphorylation of IκB (Palmieri *et al.*, 2020). After phosphorylation of IκB, the IκB/NF-κB complex dissociates, and the transcription factor NF-κB is transported to the nucleus, where it activates transcription of NF-κB-dependent genes. However, activated NF-κB not only triggers effector transcription of genes such as TNF, but also includes feedback that suppresses the LPS-activated signalling cascade. Two factors are considered the main regulators of NF-κB activation: A20, which inhibits IKK activation, and IκBα, which forms a stable complex with NF-κB and thus fixes it in the cytoplasm (Liu *et al.*, 2022; Volkova *et al.*, 2023). Since elevated concentrations of TNF pose a danger to the body, there are additional mechanisms for regulating its production. Thus, AU-rich regions within the untranslated 3'-terminal region of TNF mRNA provide reduced mRNA stability and are involved in suppression of TNF translation (Kashfi *et al.*, 2021). In addition, negative regulation of TNF production is also carried out with the involvement of secreted factors such as IL-10 and TNF itself (Fig. 2).

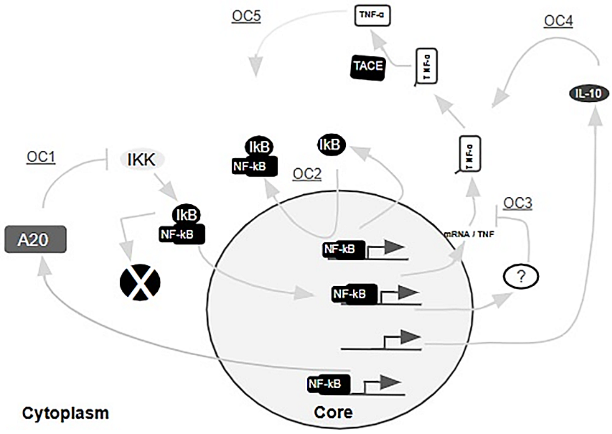

Figure 2. System for regulating TNF production in response to LPS macrophage stimulation
Source: compiled by the author

The LPS-activated signalling pathway branches out at the stage of binding the adapter protein to the receptor complex. Activated TLR4 is able to bind the TRAM/TRIF adapter complex, which triggers a signalling cascade leading to activation of the transcription factor IRF3 and to weaker and later activation of NF-κB (Nakagome & Nagata, 2024). Macrophages recognise LBP-bound LPS by a complex receptor complex that includes CD14, TLR4, and MD2. The TLR4-activated signalling cascade leads to NF-κB activation, which, in turn, enables TNF transcription. Feedback loops (FL) that regulate TNF-α production at the level of the signalling pathway and transcription regulation (FL1 and FL2), at the level of mRNA stability regulation (FL3), and involving secreted TNF-α and IL-10 factors (FL4 and FL5). Although the signal transmission system activated by a single factor remains complex due to the presence of branching signalling pathways and feedbacks, the situation in the body is even more diverse, since the causes of exposure to the cell come from several factors that can cooperate at once. A similar situation is described for IFN, which enhances the macrophage response to LPS (Li *et al.*, 2024). In addition, a similar interaction was shown for the combination of IL-4 and LPS (Muñoz *et al.*, 2020). Thus, cytokines of both Th1 and Th2 types can enhance the antibacterial response of macrophages (Scopelliti *et al.*, 2021). The interaction of multiple cytokines is even more important for anti-inflammatory factors. Cytokines and hormones involved in anti-inflammatory processes often have multifunctional properties and play an important role in the development and maintenance of homeostasis. One of the central roles in maintaining homeostasis belongs to glucocorticoids, which, in addition, are involved in embryonic development and suppression of inflammatory responses (Wu *et al.*, 2021). Similarly, TGF-β is actively involved in embryonic development and regulates fundamental processes such as cell proliferation, differentiation, and migration (Lin *et al.*, 2020). Thus, for effective action in pathological situations, such multifunctional factors must interact with each other to enhance the necessary functions and inactivate each other's potentially dangerous properties. The interaction of anti-inflammatory factors was shown for IL-4 and glucocorticoids in the case of suppression of inflammation, while dexamethasone reduced the level of TNF-α production, and IL-4 suppressed the bactericidal activity of macrophages. At the level of regulation of the expression of individual molecules, dexamethasone significantly enhances the production of CCL18, a CCR3 receptor antagonist (Zhang *et al.*, 2020; Liu *et al.*, 2022). A synergistic effect of IL-4 and dexamethasone was also observed when the expression of the macrophage mannose receptor CD163 (Hirani *et al.*, 2022). In addition to their synergistic effects, dexamethasone and IL-4 are antagonists of macrophage production of various components of the extracellular matrix and enzymes for its rearrangement.

The analysis confirmed that macrophages are a highly heterogenic population of cells that perform key functions in protecting the body from pathogens, regulating inflammatory processes, repairing tissues, and maintaining tissue homeostasis. Their functional diversity is determined by the microenvironment, which includes cytokines, pathogen-associated signals, and metabolic factors, as emphasised by T. Gauthier *et al*. (2023). Research by M.D. Gans & T. Gavrilova (2020) demonstrated that the involvement of macrophages in the development of chronic inflammation is related to their ability to respond to microenvironment signals and modulate the immune response. According to M. Locati *et al*. (2020), macrophages are characterised by a high level of functional diversity and can form a wide range of phenotypes in response to microenvironment signals. Integration of various signalling pathways ensures that cells adapt to changing tissue environment conditions. A.S.M. Moin *et al*. (2021) described activation using the M1/M2 polarisation model, according to which M1 macrophages are characterised by pronounced pro-inflammatory activity, while M2 macrophages are associated with tissue repair processes and anti-inflammatory regulation. However, the present study showed that this classification does not fully reflect the complexity of the functional states of macrophages in vivo. Research by G.A. Pizzurro & K. Miller-Jensen (2023) demonstrated that macrophage activation is a continuous spectrum of functional states, rather than a set of clearly separated phenotypes. Systemic analysis of macrophage gene expression in various biological contexts has shown the existence of numerous activation programmes that are formed under the influence of microenvironment signals and intracellular regulatory networks. This showed that the functional states of macrophages are determined by complex molecular interactions, and not just by the classical polarisation model. Integrative analysis of macrophage transcriptome profiles in various tissues and inflammatory conditions confirms this concept. In particular, D.E. Sanin *et al*. (2022) identified several conservative transcription programmes that can be grouped into four main functional trajectories: phagocytic, inflammatory, antimicrobial, oxidative stress-related, and tissue remodelling programmes. These trajectories reflect the stages of functional differentiation of macrophages and highlight the role of monocyte-derived macrophages in shaping their functional diversity during inflammation.

An important factor determining the functional state of macrophages is their metabolism. Metabolic reprogramming is necessary to maintain the cellular response to external stimuli and largely determines the functional phenotype of macrophages, as noted by T. Gauthier *et al*. (2023). Classically activated macrophages preferentially use aerobic glycolysis, which provides the synthesis of pro-inflammatory cytokines and antimicrobial factors, while alternatively activated macrophages are characterised by a more active use of oxidative phosphorylation. This study showed that the metabolic regulation of macrophages is much more complex. In turn, Y. Xia *et al*. (2022) demonstrated that transforming growth factor β (TGF-β) can induce a specific functional state of macrophages that does not fully correspond to either the M1 or M2 phenotype. Exposure to TGF-β stimulates glycolysis in macrophages by activating the mTOR-c-MYC signalling pathway and increasing the expression of the glycolytic enzyme PFKL, while inhibiting the production of pro-inflammatory cytokines through SMAD3-dependent transcription regulation. This suggests that metabolic and inflammatory signalling pathways in macrophages can be regulated independently. Impaired metabolic and immune regulation of macrophages also plays an important role in the development of metabolic and inflammatory diseases. Based on the conclusions of A.S.M. Moin *et al*. (2021), patients with obesity and type 2 diabetes exhibit an increase in markers of both M1- and M2-type macrophage activation, which indicates a mixed profile of their activation. Such dysregulation can contribute to the development of chronic inflammation. Therefore, macrophage activation is determined by the complex interaction of transcription programmes, metabolic processes, and microenvironment signals. Therefore, macrophages should be considered as cells with dynamic functional states formed in response to specific tissue and inflammatory signals.

CONCLUSIONS

The results of the analysis showed that the functional state of macrophages is determined by the complex interaction of signalling pathways that are activated under the influence of various microenvironment factors. These factors include cytokines, hormones, molecular patterns of pathogens, and other signals that shape specific cellular environment conditions. The complex action of these factors ensures the coordination of cellular responses aimed at maintaining immune homeostasis, regulating inflammatory processes, and adapting cells to environmental changes. Glucocorticoids, TGF-β, and cytokines Th1 and Th2 have been shown to exhibit both synergistic and antagonistic effects in regulating macrophage functional activity. Such interactions determine the level of inflammatory response, tissue repair processes, and the participation of macrophages in the pathogenesis of various diseases, in particular, tumourigenic and inflammatory processes. It was found that the balance between pro-inflammatory and anti-inflammatory signals is an important factor in maintaining the normal functioning of the immune system and preventing the development of pathological changes in tissues. The study confirmed that the development of various macrophage phenotypes is determined not only by a combination of microenvironment factors, but also by the sequence of their effect on the cell. The data obtained support the concept of macrophage plasticity, according to which these cells can change their phenotype within a continuum of functional states depending on changes in the microenvironment. Such plasticity ensures the adaptability of the immune response, but simultaneously can contribute to the development of pathological processes. Further study of the mechanisms of signalling interaction pathways in macrophages is important for understanding the molecular basis of immune regulation and may contribute to the development of new therapeutic approaches for the treatment of inflammatory and oncological diseases. Deepening knowledge about the molecular mechanisms of macrophage polarisation and their functional plasticity is essential for the development of contemporary immunology and biomedicine, and for the creation of new targeted therapy strategies aimed at regulating the activity of these cells in various pathological conditions.

ACKNOWLEDGEMENTS

None.

FUNDING

None.

CONFLICT OF INTEREST

None.

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Сигнальні шляхи макрофагів
та їх регуляція факторами клітинного мікрооточення

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Анотація. Макрофаги є ключовими клітинами вродженого імунітету, що відіграють важливу роль у регуляції запальної відповіді, процесах репарації тканин та розвитку різних патологічних станів, зокрема онкологічних і хронічних запальних захворювань. Функціональна активність макрофагів значною мірою визначається сигналами мікрооточення, включаючи цитокіни, гормони, фактори росту та патоген-асоційовані молекулярні структури. Метою роботи було узагальнення сучасних наукових даних щодо молекулярних механізмів регуляції функціональної активності макрофагів під впливом факторів мікрооточення та аналіз взаємодії сигнальних шляхів, що визначають їхню поляризацію і функціональну пластичність. У роботі проаналізовано результати сучасних досліджень, присвячених регуляції сигнальних каскадів макрофагів за участю цитокінів Th1- та Th2-типу, глюкокортикоїдів, трансформувального фактора росту β (TGF-β), а також сигнальних шляхів, активованих патоген-асоційованими молекулярними структурами. Показано, що активація рецепторних комплексів, зокрема TLR4, запускає складні сигнальні каскади, пов'язані з активацією транскрипційного фактора NF-κB та MyD88-залежних механізмів передачі сигналу. Встановлено, що взаємодія різних сигнальних молекул може призводити як до синергічних, так і до антагоністичних ефектів, що впливають на експресію генів, пов'язаних із запальною відповіддю, клітинною адгезією та метаболізмом ліпідів. Отримані узагальнені дані підтвердили концепцію континууму функціональних станів макрофагів, згідно з якою ці клітини здатні змінювати свій фенотип залежно від умов мікрооточення. Ці результати підкреслили важливість вивчення механізмів взаємодії між різними сигналами мікрооточення для розуміння регуляції макрофагальних функцій. Практична цінність роботи полягає у систематизації сучасних уявлень про механізми регуляції макрофагів, що може бути використано для подальших досліджень молекулярних основ імунної відповіді, а також для розробки нових підходів до терапії запальних, аутоімунних та онкологічних захворювань

Ключові слова: імунна відповідь; TGF-β; цитокіни; пухлина; фактори росту

Received: 04.10.2025 Revised: 23.01.2026 Accepted: 26.02.2026 Published: 07.04.2026

BIOLOGICAL SYSTEMS: Theory and Innovation

ISSN 2706-8382
e-ISSN 2706-8390

Journal homepage: https://bioscience.com.ua/en
Vol. 17, No. 1, 2026, 78-90

UDC 633.11:577.4:519.876

DOI: 10.31548/biologiya/1.2026.78

Correlation and path analysis of morphological traits in a bread wheat collection using Structural Equation Modelling

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Abstract. The aim of this study was to investigate the structural determinants of grain yield per plant in bread wheat (*Triticum aestivum* L.) and to assess the contributions of morphological and yield-related traits. Descriptive statistics, Pearson's correlation analysis, path coefficient analysis, and Structural Equation Modelling (SEM) were employed. A total of 53 genotypes from the bread wheat collection of the Genetic Resources Institute (Baku, Azerbaijan) were evaluated for grain weight per spike, thousand-kernel weight (TKW), grains per spike (GPS), plant height (PH), and main spike length (SL). Analysis revealed that grain yield per plant (GWPP) was primarily influenced by numerical (GPS, $r = 0.47$) and mass-related (TKW, $r = 0.29$) traits, whereas architectural traits (PH, SL) contributed mainly indirectly. SEM showed that grain weight per spike (GWPS) acted as a central mediating variable, channelling the effects of GPS and TKW to overall yield. The mass/sink axis (TKW → GWPS) exhibited a stronger direct effect (standardised coefficient = 2.18) than the architectural axis (0.41), while architectural traits primarily supported sink capacity indirectly. The final model demonstrated excellent fit ($\chi^2(3) = 1.26$, $p = 0.74$; Comparative Fit Index = 1.00; Root Mean Square Error of Approximation = 0.00; Standardised Root Mean Square Residual = 0.036), supporting the biological interpretability of the observed trait relationships. These results provide practical insights for plant breeders and geneticists seeking to select high-yielding wheat genotypes under

Suggested Citation:
Eyyubova, N., Sadiqov, H., Sadigova, S., & Gasim, N. (2026). Correlation and path analysis of morphological traits in a bread wheat collection using Structural Equation Modelling. *Biological Systems: Theory and Innovation*, 17(1), 78-90. doi: 10.31548/biologiya/1.2026.78.

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controlled field conditions and can be utilised in wheat breeding programmes to optimise strategies for improving bread wheat production

Keywords: *Triticum aestivum* L.; grain yield; classical framework; chemical treatments; source-sink dynamics

INTRODUCTION

Investigating the correlation and path relationships of morphological traits in bread wheat using Structural Equation Modelling (SEM) is essential for understanding the complex interactions among yield components, including grain number, grain weight, and plant architecture. Such analysis enables the identification of direct and indirect effects of mass-related, numerical, and architectural traits on overall productivity, providing insights into which characteristics most strongly influence yield formation. By quantifying these relationships within diverse wheat genotypes, breeders can prioritise traits that contribute most effectively to grain yield, optimise selection strategies, and improve the efficiency of breeding programmes. Additionally, SEM allows for the integration of multiple traits into a coherent model, revealing hidden patterns and mediation effects that traditional correlation analyses may overlook, thereby supporting data-driven decision-making in wheat improvement under specific environmental and agronomic conditions. The theoretical foundation of this study rests on two complementary axes.

The first is the numerical axis, represented mainly by the number of grains per spike (GPS). L. Vicentin *et al.* (2024) investigated the contribution of the numerical component to yield variability in bread wheat and established that a large proportion of yield differences originated from this axis. T. Fischer *et al.* (2024) examined the impact of numerical traits on overall grain production and highlighted their critical role in genotype selection. K. Wang *et al.* (2023) analysed the effects of limited photosynthetic supply on mass per sink capacity and demonstrated its importance for maintaining yield stability under stress conditions. In research C. Zhang *et al.* (2022) explored the physiological processes underlying average grain mass (TWK), including carbon allocation, assimilate transport, and endosperm development during grain filling. R.B. Flavell (2023) studied the architectural axis and its regulation of source-sink balance, showing how synergistic operation of numerical and architectural traits enhanced grain weight per spike (GWPS) and grain yield per plant (GWPP). X.-L. Wu *et al.* (2022) interpreted yield formation within the classical source-sink framework, emphasising the equilibrium between assimilate supply and sink capacity during pre- and post-flowering phases. In particular, T.O. Milkessa (2022) demonstrated that grain yield was frequently limited by sink capacity during grain filling, indicating the necessity to improve both number-related (GPS, NoSPS) and mass-related traits (TWK) simultaneously to enhance overall GWPP through the mediating effect of GWPS. In the study, T.H. Borkhatariya *et al.* (2025) investigated the relationships between yield and yield-related traits in bread wheat using correlation and path coefficient analysis. Furthermore, they identified key traits, such as biological yield and number of effective tillers per plant, which had significant direct effects on grain yield. In addition, they evaluated the influence of plant height and spike length on overall productivity, determining their indirect effects through key yield components. Moreover, the study emphasised the importance of selecting genotypes with optimised combinations of numerical and mass-related traits to enhance stable grain yield under diverse environmental conditions. The study identified gaps including limited genotype coverage, insufficient multifactor analysis of numerical and mass-related yield components, inadequate evaluation of architectural traits under diverse environments, lack of multi-season and multi-location trials, and insufficient integration of modern Structural Equation Modelling (SEM) approaches, restricting the predictive capacity for yield stability and the selection of high-performing bread wheat cultivars.

The objective was to examine the factors influencing GWPP in bread wheat (*Triticum aestivum* L.) and to assess the roles of various morphological and yield-associated traits. Accordingly, in this study, biologically justified alternative SEM pathway models were constructed, evaluated through indices such as Comparative Fit Index (CFI), Tucker-Lewis Index (TLI), Root Mean Square Error of Approximation (RMSEA), and Standardised Root Mean Square Residual (SRMR), and interpreted based on standardised coefficients and bootstrap validation. Given the single-environment nature of the dataset, the objective was to explore the internal structural relationships among yield components rather than to make broad, environment-general breeding recommendations.

MATERIALS AND METHODS

The experiment was conducted during the 2025 spring growing season. The experiment was carried out in the experimental field of the Azerbaijan State Genetic Resources Institute, which is officially designated for scientific research on cereal crops. A total of 53 bread wheat varieties 'Oyxan', 'Ekinchi-84', 'Tereqqi', 'Murov', 'Qaudio', 'Mirbeshir-128', 'Romanna', 'Ferehim', 'Midas', 'Nogal', 'Yasavul', 'Olimp', 'Nurlu-99', 'Khamsa', 'Qualite', 'Deabar', 'Chempion', 'Bayraqdar', 'Azeri', 'Qobustan', 'Onur', 'Yuka', 'Lider', 'Shefeq-2', 'Mirastar', 'Fatime', 'Shahbugda', 'Xlebodarka', 'Farandol', 'Khazri', 'Renan', 'Atilla', 'Batko', 'Ugur', 'Baqrat', 'Auradur', 'Leyaqetli-80', 'Guneshli', 'Qizilbughda', 'Vusal', 'Metin', 'Kroshka', 'Zirve-85', 'Esgeran', 'Stan-Krasnodar', 'Gallio', 'Murov-2', 'Balaton', 'Seba-2', 'Qirmizigul', 'Tunc', 'Maruzio', 'Gyrmyzy bughda' were used as experimental material. Each genotype was grown in a single-row plot with a single replication (one plot per genotype) under uniform management. The field was prepared using conventional tillage operations applied uniformly across the entire experimental area prior to sowing. All genotypes were sown on the same date, at the same seeding rate, row spacing, and planting depth to eliminate variation in plant density and early establishment. Basal mineral fertilisation was applied evenly across the field according to regional agronomic recommendations for bread wheat, ensuring equal nutrient availability for all plots. Irrigation was carried out uniformly across the experimental site based on crop water requirements and prevailing climatic conditions, preventing differential moisture stress among genotypes.

Weed control was maintained through identical mechanical and/or chemical treatments applied simultaneously to the whole field area. Preventive plant protection measures against common wheat pests and diseases were implemented uniformly, and no genotype-specific chemical treatments were applied. No thinning or selective removal of weaker plants was performed within individual plots, in order to avoid artificial alteration of genotype performance. All plots were managed under the same environmental and agronomic conditions without differential interventions. These standardised practices ensured that observed differences among genotypes reflected genetic variability rather than variation in management conditions. This experiment represents a single-environment evaluation (one growing season and one location). The un-replicated (single-replication) structure and lack of multi-environment testing limit the generalisability of estimated associations; thus, the SEM was used primarily to explore internal trait relationships within the studied germplasm set.

The study was conducted in full compliance with the ethical principles of the Convention on Biological Diversity (1992), and all established ethical requirements were strictly observed. The plant materials used in this study were part of the official wheat germplasm collection of the Genetic Resources Institute, and no special ethical approval was required. Descriptive statistics (mean, standard deviation, and coefficient of variation) were computed for all morphological and yield-related traits. Pearson's correlation coefficients were calculated to quantify pairwise associations among traits. To determine the magnitude and direction of both direct and indirect effects of the studied traits on GWPP, path analysis was performed following the classical framework proposed by S. Wright (1921) and further developed by C.C. Li (1975). Given the moderate sample size ($n = 53$), particular emphasis was placed on model parsimony and biological interpretability. The SEM was therefore specified with a limited number of latent constructs and theoretically justified pathways to reduce the risk of overparameterisation. Model fit indices were interpreted cautiously, recognising that very high values (e.g., CFI ≈ 1.00, RMSEA ≈ 0.00) may partly reflect low degrees of freedom rather than perfect biological representation. All statistical computations, including descriptive statistics, correlation analysis, and path coefficient estimation, were carried out using the Jamovi software (version 2.4.14) and Python (version 3.12.4) environments.

RESULTS

First, all candidate variables were subjected to a preliminary examination using descriptive statistics and a correlation matrix. Subsequently, a biologically justified multiple path model (MPM) was constructed, and comparisons were performed based on fit indices (χ^2, CFI, TLI, RMSEA, SRMR), parsimony indicators (Parsimony Normed Fit Index (PNFI), Akaike Information Criterion/ Bayesian Information Criterion (AIC/BIC)), and standardised coefficients/Bootstrap GA-based comparisons. During this screening process, the variables NoPT (number of productive tillers), NoSPS (number of spikelets per spike), and GPS (number of grains per spikelet) were evaluated and tested in structural blocks in different schemes. However, when making the final (best fit-simplest structure) selection, adding these variables to the model as free paths (i) did not increase global fit, (ii) did not independently and consistently increase the explained variance contribution compared to the basic components already present in the model (especially GWPS and TWK), and (iii) weakened parsimony in the context of the sample size (n = 53). The selected final model provides excellent global fit and high interpretability by focusing on the relationship between mass/sink capacity captured through the information content GWPS and TWK and GWPP. This choice is based on two principles: (i) The principle of mediation – the effect of numerical components (NoSPS, GPS) is primarily reflected in yield through the GWPS channel; therefore, repeatedly introducing the same information into the model both directly and indirectly creates unnecessary freedom and uncertainty. (ii) The principle of parsimony – removing paths that do not increase fit in a small/medium sample (n = 53) increases the stability of the coefficients and the reliability of the inferences. However, the descriptive statistics and bivariate relationships of the NoPT-NoSPS-GPS variables are included in the report; their roles in the theoretical framework (representing numerical capacity and spike architecture) are discussed in the discussion section. Thus, while theoretical integrity is preserved, the empirically strongest and simplest structure is carried over to the results section. The descriptive statistics obtained at the beginning of the study in Table 1 reveal both the central tendencies and the distributional patterns of the variables, guiding how subsequent modelling steps should be designed.

Table 1. Descriptive statistics

	GWPP	TKW	GPS	PH	LoS
Mean	6.84	49.3	45.7	103.6	11.3
Median	6.58	49	45.4	102.8	10.3
Maximum	20.3	62	70.8	126	38.9
Minimum	2.60	40	32	83.4	6.8
Std. Dev.	2.79	5.38	7.50	9.50	5.03
Coef.Var	40.8	10.9	16.4	9.16	44.5
Skewness	2.19	0.28	0.72	0.07	4.14
Kurtosis	11.3	2.89	4.07	2.59	20.8
Jarque-Bera	192.6	0.724	7.074	0.413	855.1
Probability	0.000	0.696	0.029	0.813	0.000
Observations	53	53	53	53	53

Source: compiled by the authors

GWPP: The mean is 6.84 g plant^{-1}, the median is 6.58, and the range is 2.60-20.30. With a standard deviation of 2.79 and CV of 40.8%, a high dispersion is observed. The skewness = 2.19 and kurtosis = 11.3 measures indicate that the distribution is distinctly right-skewed and heavy-tailed. The Jarque-Bera = 192.6 ($p < 0.001$) result confirms a significant deviation from normal distribution. TKW: The mean is determined to be 49.3 g 1,000 grains^{-1}, the median is 49.0, and the range is 40-62. Standard deviation = 5.38 and CV = 10.9% indicate low variability. Skewness = 0.28 and kurtosis = 2.89 values show that the distribution is close to normal; Jarque-Bera = 0.724 ($p = 0.696$) confirms that this fit is statistically supported. GPS: The mean is 45.7, the median is 45.4, and the range is 32-70.8. With a standard deviation of 7.50 and a CV of 16.4%, a moderate level of dispersion is observed. Skewness = 0.72 indicates a moderately positive tail, and kurtosis = 4.07 indicates slight peakedness. The Jarque-Bera = 7.074 ($p = 0.029$) value indicates a limited deviation from the normal form. PH: The mean is 103.6 cm, the median is 102.8 cm, and the range is 83.4-126 cm. With a standard deviation of 9.50 and CV = 9.16%, the lowest dispersion is observed in this variable. The measures skewness = 0.07 and kurtosis = 2.59 indicate that the distribution is symmetric and close to normal; Jarque-Bera = 0.413 ($p = 0.813$) confirms that the fit to the normal distribution is acceptable. LOS (ear length). The mean is recorded as 11.3 cm, the median as 10.3 cm, and the range as 6.8-38.9 cm. With a standard deviation of 5.03 and a CV of 44.5%, the highest heterogeneity is observed in this variable. Skewness = 4.14 and kurtosis = 20.8 indicate a pronounced right-tail and heavy-tail structure. The Jarque-Bera = 855.1 ($p < 0.001$) result confirms a strong deviation from normal distribution. Following descriptive statistics, bivariate relationships between variables were examined using the Pearson correlation matrix in Figure 1, where coefficient magnitudes are categorised according to conventional thresholds (≈0.10-0.29 weak; ≈0.30-0.49 moderate; ⩾0.50 strong).

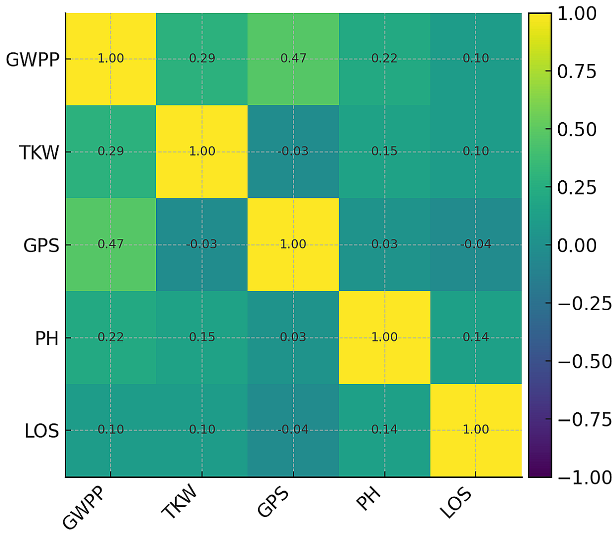

Figure 1. Correlation matrix heatmap

Source: compiled by the authors

GWPP was found to have a moderate and positive relationship with GPS ($r = 0.471$). This result suggests that yield is significantly influenced by the numerical component (number of grains per head). A weak-moderate positive relationship was found with TWK ($r = 0.286$); it is understood that the increase in average grain mass contributes to yield, but to a more limited extent compared to GPS. Relationships with architectural indicators are weak: PH-GWPP $r = 0.218$, LoS-GWPP $r = 0.102$; this pattern implies that architecture reflects on yield more through indirect channels. It has been determined that the relationship between TKW and GPS is negligible ($r = -0.028$). This finding indicates that no significant "compensation/trade-off" signal has emerged between the numerical and mass components under these materials and conditions, suggesting that the two components can move independently. The weak positive relationships between TKW and PH ($r = 0.153$) and LoS ($r = 0.103$) suggest that mass capacity is only moderately correlated with architecture. GPS has a very low correlation with architectural indicators (PH-GPS $r = 0.025$, LoS-GPS $r = -0.037$). This indicates that the processes determining spikelet/floret dynamics and fertility can operate independently of the measured architectural indicators. On the other hand, GPS's moderate positive correlation with GWPP (r = 0.471) confirms its effect on yield. A weak positive relationship was found between the two architectural traits (r = 0.139). This magnitude suggests that architectural components, while changing together, are not powerful enough to explain yield variation on their own and should be considered as control or indirect effects in structural models. No high correlation ($|r| \geq 0.85$) was found between predictors. In particular, the fact that the TWK-GPS relationship is close to 0 indicates that the risk of multicollinearity is low when both numerical and mass components are included in the model simultaneously. This profile statistically supports the separation of the effects of the mass axis (TWK/GWPS) and the numerical axis (GPS) in road analysis. The pattern in the matrix shows that productivity (GWPP) is primarily driven by numerical (GPS) and mass (TWK) components, while architectural indicators (PH, LoS) exhibit weak singular relationships. Therefore, it is appropriate to model the yield flow in the final structural design along the GWPS/TKW axis and based on the assumption of the indirect effect of GPS; architectural variables should be considered in an indirect/conditional role when there is a theoretical justification. Following descriptive statistics and correlation patterns, a Structural Equation Model was estimated to evaluate the extent to which the theoretical flow (architecture → numerical/mass components → ear mass → yield) was supported by the data. The goodness-of-fit results for this model are presented in Table 2.

Table 2. Model fit indices for the estimated SEM structure

Model tests			
Label	**χ^2**	**df**	**p**
User model	1.26	3	0.740
Baseline model	25.08	10	0.005

Source: compiled by the authors

The chi-square test for the user model, $\chi^2(3) = 1.26$, $p = 0.740$, indicates that there is no significant difference between the observed covariance matrix and the covariance predicted by the model. This result indicates that the absolute fit is very good and that the model contains at least 3 over-specified constraints (parsimony). The baseline model provided for comparison was significantly rejected with $\chi^2(10) = 25.08$, $p = 0.005$; the data does not support the assumption that the variables are independent of each other. This dual table also implies that the relative fit indices (CFI, TLI/Non-Normed Fit Index (NNFI), Bollen's Incremental Fit Index (IFI), Normed Fit Index (NFI), Relative Fit Index (RFI)) will be high; indeed, CFI reaches 1.00 as required by the formula (when χ^2_user – df_user ≤ 0, the numerator becomes 0). Furthermore, because χ^2 is smaller than df, Root Mean Square Error of Approximation (RMSEA) is expected to be very close to 0. In summary, the model tests results show that the established structure explains the data successfully and parsimoniously; therefore, in the next step, the standardised path coefficients and indirect/total effects can be interpreted with confidence. The detailed fit indices, including SRMR and RMSEA values, are summarised in Table 3.

Table 3. Fit indices

95% confidence intervals				
SRMR	**RMSEA**	**Lower**	**Upper**	**RMSEA p**
0.036	0.000	0.000	0.163	0.000

Source: compiled by the authors

The Standardised Root Mean Square Residual (SRMR) = 0.036 indicates that the average size of standardised residuals is low and that the ≤0.08 (preferably ≤0.05) threshold is clearly met; this implies that the model captures the covariance structure at a detailed level. The RMSEA = 0.000 shows that the model error is well below the "close fit" threshold (≈0.05-0.06), and the RMSEA p(close) = 0.777 indicates that the "close fit exists" hypothesis cannot be rejected – strong evidence in favour of a close fit. The lower bound of the RMSEA 95% CI [0.000, 0.163] touching 0 indicates that the point estimate is very low, while the width of the upper bound may reflect uncertainty due to the small df. When considered together, the SRMR and RMSEA results in Table 3 confirm that the model explains the data at a very good/close fit level, and that the parameter (path coefficient) interpretations can be made with confidence. It should be noted that extremely high incremental fit indices (e.g., CFI = 1.00, RMSEA = 0.00) are not interpreted here as evidence of biological perfection. In small or moderately sized samples with low degrees of freedom, such values may arise from model parsimony and constrained structure. Accordingly, model evaluation focused on consistency with biological theory rather than on numerical maximisation of fit indices (Table 4).

Table 4. User model versus baseline model

Fit measure	Model	Reference/Cutoff	Assessment
CFI	1.000	≥0.95 good (≥0.90 acceptable)	Excellent
TLI	1.386	≥0.95 good	Excellent (interpreted as ≈1.00)
NNFI	1.386	≥0.95 good	Excellent (≈1.00)
RNI	1.116	≥0.95 good	Excellent (≈1.00)
NFI	0.950	≥0.95 good (≥0.90 acceptable)	Good (at threshold)
RFI	0.833	≥0.90 preferred	Moderate/Below preferred
IFI	1.079	≥0.95 good	Excellent (≈1.00)
PNFI	0.285	No hard cutoff; higher = more parsimonious (≈ >0.50 moderate)	Low parsimony (few df)

Note: RNI – Relative noncentrality index
Source: compiled by the authors

After confirming absolute and local fit using chi-square, SRMR, and RMSEA, additional incremental (relative) fit indices were examined to evaluate how much the user model improved compared to the baseline model, as summarised in Table 4. The incremental/relative indices indicate that the model fits very well compared to the independence model: CFI = 1.000 is excellent; TLI/NNFI and IFI exceeding 1 are due to software rounding and are interpreted in practice as ≈1.00 (perfect fit). NFI = 0.950 fully meets the good threshold value. Although RFI = 0.833 is relatively low, this index is sensitive to df and can be explained by the small number of constraints. PNFI = 0.285 indicates low parsimony (few constraints/few df), which is not a fit issue but rather a measure of the model's simplicity. The coefficients in the path diagram (factor loadings, error terms, and direct/indirect effects) were interpreted on a numerical basis (Grace, 2009). It was determined that the latent structure of Endg1 was scaled with a loading of 1.00 using GWPP and therefore the error term for GWPP was 0.00. The connection of Exgn1, representing the mass/sink axis, with TWK was ≈ 1.00 and the indicator error term was at the level of ε_TKW ≈ 1.02. The architectural/vigor axis Exgn2 was carried by PH with a loading of 0.54 (ε_PH ≈ 0.71) and LoS with a loading of 0.26 (ε_LoS ≈ 0.93). This pattern indicates that PH is more strongly represented on the architectural axis than LoS (0.54 > 0.26) and that the relative error share in the LoS measurement is higher (0.93 > 0.71). For the numerical efficiency indicator at the head level, an error term ε_GPS ≈ 1.05 was recorded; it was also observed that GPS has out-of-model determinants (Ochagavía *et al.*, 2018; Slafer *et al.*, 2023; Wang *et al.*, 2023). Overall, these results confirm that the model exhibits strong relative fit, justifying the interpretation of the standardised path coefficients with confidence. The final structural model, depicting the direct and indirect relationships among yield-related traits, is presented in Figure 2. The strong association between the mass/sink axis and yield (Exgn1 → Endg1 = 2.18) indicates that, within this dataset, maintaining kernel weight during the grain-filling period represents a dominant structural component of yield formation. In this single-environment evaluation, the observed dominance of the mass/sink-related axis is consistent with the recognised importance of kernel weight maintenance. However, specific agronomic recommendations (e.g., irrigation management or late-season nitrogen strategies) cannot be inferred from the present design and should be explicitly tested in multi-environment and/or managed-stress experiments. The positivity of Exgn1 → GPS conveys the message that suppressing TKW is not mandatory while increasing grain number; it is understood that the target of "GPS↑ + TKW ≈ stable" is achievable with appropriate genotype × environment arrangements. Exgn2 → Based on the Endg1 = 0.41 result, the architecture should be maintained within a yield-supporting range: Considering the strong load of 0.54 for PH, sufficient vigour should be ensured without increasing the risk of lodging. Due to the weak load of 0.26 for LoS and the error ε_LoS ≈ 0.93, monitoring spike length as a secondary/auxiliary indicator should be sufficient. Ultimately, the strategy that most rapidly reflects an increase in GWPP is "supporting GPS while preserving (and if possible increasing) TWK" and managing architecture at a level that facilitates this process (Zhang *et al.*, 2020; Wu *et al.*, 2022; Fang *et al.*, 2022). From a methodological perspective, the moderate sample size necessitates cautious interpretation of the SEM results. Although the model was intentionally specified in a parsimonious manner, the possibility of capitalising on sample-specific covariance patterns cannot be entirely excluded. Accordingly, the SEM presented here should be regarded as an exploratory representation of yield-component structure and requires confirmation using larger and replicated datasets. The study is based on a single location, a single growing season, and one replication per genotype. Consequently, the magnitude of correlations and SEM pathways may differ under alternative environmental conditions, management regimes, or stress scenarios. The present findings should therefore be regarded as exploratory and hypothesis-generating, providing a basis for future validation in multi-environment studies.

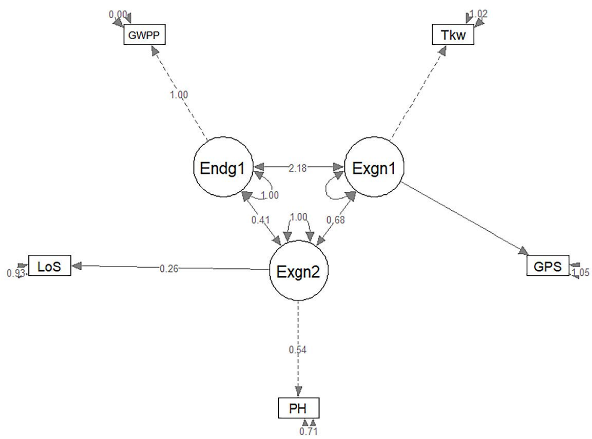

Figure 2. Path diagram of the SEM depicting direct and indirect relationships among variables among key morphological and yield-related traits in bread wheat

Source: compiled by the authors

DISCUSSION

In the results of the present study, it was determined that the main components influencing yield formation in the evaluated bread wheat genotypes were grain weight per spike and thousand-kernel weight, which had a strong direct positive effect on grain yield per plant, whereas architectural traits (plant height and spike length) acted as secondary factors influencing yield mainly indirectly through numerical and mass components. These findings are consistent with M. Barman *et al.* (2020), who reported a significant contribution of morpho-physiological traits and grain Fe and Zn content to yield and its components, while A. Baye *et al.* (2020) highlighted the direct and indirect effects of genotypic and phenotypic traits on grain yield in advanced wheat lines, confirming the role of both mass and numerical components in productivity. Furthermore, A.A. Ezici *et al.* (2022) demonstrated that morphological and quality characteristics determine yield variability, which aligns with the relationships observed in the current study between mass and grain number indicators. Overall, all three studies support that the direct effect of mass-related components on yield is dominant, whereas numerical and architectural traits play auxiliary or indirect roles. In the study, G. Kour *et al.* (2025) assessed genetic variability and applied correlation and path analysis to determine the direct and indirect influences of morphological traits on grain yield. Furthermore, they demonstrated that certain architectural and numerical traits contributed differently to overall productivity, providing insights for targeted selection. S. Mahpara *et al.* (2024) analysed diverse wheat genotypes to quantify the effects of spike length, plant height, and grain weight on grain yield. In addition, they established which traits could be most efficiently manipulated to enhance yield performance in breeding programmes. In the article, A.G. Rajput *et al.* (2025) conducted correlation and path coefficient analysis in bread wheat to identify the relative contributions of morphological traits to productivity. Furthermore, they reported that both direct and indirect pathways of certain traits significantly affected grain yield, highlighting the complexity of yield formation. M. Yadav *et al.* (2025) examined genetic parameters and performed correlation and path analysis for yield and yield-contributing traits. Moreover, they provided recommendations for breeders on prioritising specific traits to achieve higher and more stable wheat yields.

In the present study, the dominant contribution of mass-related components, specifically grain weight per spike and thousand-kernel weight, to overall yield was confirmed, with architectural traits such as plant height and spike length exerting mainly indirect effects through numerical and mass traits. These findings are consistent with M.I. Ullah *et al.* (2021), who reported that grain yield in bread wheat lines is primarily influenced by mass and numerical traits, and that direct and indirect correlations among yield components are critical for yield improvement. Similarly, I. Havryliuk & H. Kovalyshyna (2025) and S. Arshad *et al.* (2025) showed that in soft winter wheat hybrids, morphological traits of the spike and productive components of the ear are strongly associated with grain yield, supporting the conclusion that mass and spike-related traits play a dominant role, while architectural features act indirectly. Collectively, these studies reinforce that maintaining mass-related traits is essential for yield optimisation, whereas numerical and architectural traits contribute in secondary or mediating roles.

The study demonstrated that grain yield formation in bread wheat is primarily governed by mass-related components, particularly GWPS and TKW, which exerted the strongest direct effects on GWPP. Numerical components, represented by GPS, and architectural traits, including PH and LoS, contributed predominantly through indirect pathways mediated via GWPS and TKW. Correlation and path analyses confirmed that mass and numerical traits operate largely independently, with minimal trade-off, enabling simultaneous improvement of both components. Specifically, V. Chauhan *et al.* (2025) examined seventy-five wheat genotypes and evaluated their phenotypic and genotypic performance for yield-related traits. In addition, they highlighted that seed yield per plant was strongly associated with number of grains per spike and thousand-kernel weight, providing guidance for superior cultivar selection. In the article, M. Javed *et al.* (2024) and G.P. Sharadhi *et al.* (2023) estimated heritability and genotypic variability among bread wheat genotypes and analysed correlations for yield-attributing traits. Moreover, they emphasised the traits that could be prioritised in breeding programmes to improve grain yield stability under varying conditions.

The results indicated that mass-related traits are the most influential factors in yield determination, while numerical and architectural characteristics play supporting roles. D. Zewdu *et al.* (2024) investigated the relationships among yield and yield component traits in bread wheat (*Triticum aestivum* L.) genotypes. They applied correlation and path analysis to identify key traits influencing grain yield and to quantify their direct and indirect effects. These findings highlighted the importance of prioritising breeding and agronomic strategies that enhance grain mass while maintaining spike fertility and optimal plant architecture to maximise productivity. The study provided a foundation for further research under multi-environment trials to validate the observed relationships and explore genotype × environment interactions affecting yield components.

CONCLUSIONS

The study demonstrated that wheat yield formation is governed predominantly by the interaction of numerical and mass-related traits rather than by architectural morphology. Among the examined traits, the number of GPS and the TWK jointly determined the majority of yield variation, whereas plant height (PH) and ear length (LOS) exhibited only indirect contributions through their influence on spike vigour and assimilate allocation. The SEM confirmed that GWPS functions as the central mediating variable, transmitting the effects of GPS and TWK onto overall GWPP. The quantitative results clearly emphasised that the mass/sink axis (TWK + GWPS) exerts a substantially stronger direct effect on yield compared with the architectural axis, confirming that the maintenance of sink capacity during the grain-filling phase is critical for achieving high productivity. The positive association between the mass and numerical axes suggests that simultaneous improvement in both grain number and grain mass is attainable without a strong trade-off, provided that environmental and genetic conditions are properly balanced. From a breeding perspective, the observed structure suggests that, within this germplasm and under the studied conditions, GPS and TWK-related components merit attention as primary yield-associated traits. The weak direct associations of architectural traits imply that their role is largely indirect, acting through support of sink-related processes rather than as primary drivers of yield variation. Nevertheless, the extent to which these relationships hold across years, environments, and management regimes should be confirmed using replicated multi-location trials before agronomic or breeding recommendations are generalised. The structural relationships identified here should be interpreted as hypothesis-generating and provide a basis for future SEM analyses using larger, multi-environment datasets. Overall, the study provided an integrative framework for understanding yield architecture in bread wheat, indicating that improving the balance between numerical and mass-related components of productivity may represent a more effective pathway to genetic gain than relying solely on morphological modification. These insights can inform future breeding research and generate hypotheses to be tested for yield stability in multi-environment evaluations. The results revealed that wheat yield formation depends mainly on the synergistic balance between grain number and grain mass rather than on architectural morphology. These findings, within the limits of a single-season, single-location evaluation, suggest that selection emphasis may be placed on genotypes combining higher GPS with stable TWK, but broader inference requires multi-environment validation. This study provided one of the first SEM-based insights into yield architecture in Azerbaijan's wheat germplasm. Because the experiment was conducted in one environment (single year, single location) with one replication, the results should be interpreted as exploratory and context-specific rather than broadly generalisable. A promising direction for future research is the evaluation of the stability of the identified structural relationships between yield components in multi-location, multi-factor trials, taking into account genotype and environment interactions.

ACKNOWLEDGEMENTS

The authors express sincere gratitude to the staff and administration of the Azerbaijan State Genetic Resources Institute, as well as to all participants and institutional representatives whose support and cooperation contributed significantly to the successful completion of this research.

FUNDING

None.

CONFLICT OF INTEREST

None.

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Кореляційний та шляховий аналіз морфологічних ознак у колекції м'якої пшениці за допомогою моделювання структурних рівнянь

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Анотація. Метою цього дослідження було вивчення структурних детермінант урожайності зерна з однієї рослини у пшениці м'якої (*Triticum aestivum* L.) та оцінка внеску морфологічних і продуктивних ознак. У роботі застосовано методи описової статистики, кореляційний аналіз Пірсона, аналіз шляхових коефіцієнтів і моделювання структурними рівняннями (SEM). Досліджено 53 генотипи з колекції генетичних ресурсів Інституту генетичних ресурсів (м. Баку, Азербайджан) за такими показниками: маса зерна з колоса, маса тисячі зерен (TKW), кількість зерен у колосі (GPS), висота рослини (PH) і довжина головного колоса (SL). Аналіз показав, що врожайність зерна з однієї рослини (GWPP) визначається передусім кількісними (GPS, $r = 0,47$) і масовими (TKW, $r = 0,29$) показниками, тоді як архітектурні ознаки (PH, SL) мають переважно опосередкований вплив. Моделювання SEM засвідчило, що маса зерна з колоса (GWPS) виступає центральною медіаторною змінною, через яку реалізується вплив GPS і TKW на загальну врожайність. Вісь «маса/стік» (TKW → GWPS) характеризується сильнішим прямим ефектом (стандартизований коефіцієнт = 2,18), ніж архітектурна вісь (0,41), тоді як архітектурні ознаки переважно опосередковано підтримують потенціал «стоку». Побудована модель продемонструвала відмінну відповідність даним ($\chi^2(3) = 1,26$, $p = 0,74$; CFI = 1,00; RMSEA = 0,00; SRMR = 0,036), що підтверджує біологічну інтерпретованість виявлених взаємозв'язків між ознаками. Отримані результати мають практичне значення для селекціонерів і генетиків рослин у процесі добору високоврожайних генотипів пшениці в контрольованих польових умовах і можуть бути використані для оптимізації стратегій підвищення продуктивності пшениці м'якої

Ключові слова: *Triticum aestivum* L.; урожайність зерна; класична структура; хімічні обробки; динаміка джерела-поглинача

БІОЛОГІЧНІ СИСТЕМИ: Теорія та Інновації

Науковий журнал
Том 17, № 1. 2026

Заснований у 2010 р. Періодичність випуску: щоквартально

Оригінал-макет видання виготовлено у відділі науково-технічної інформації
Національного університету біоресурсів і природокористування України

Відповідальний редактор:
О. Наумовська

Підписано до друку 26 лютого 2026 р.
Формат 70*100/16
Умов. друк. арк. 7,5
Наклад 100 прим.

Адреса редакції:
Національний університет біоресурсів і природокористування України
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BIOLOGICAL SYSTEMS: Theory and Innovation

Scientific Journal
Volume 17, No. 1. 2026

Founded in 2010. Frequency: quarterly

The original layout of the publication is made in the Department of Scientific and Technical Information of National University of Life and Environmental Sciences of Ukraine

Managing Editor:
O. Naumovska

Signed for print of February 26, 2026
Format 70*100/16
Conventional printed pages 7.5
Circulation 100 copies

Editors office address:
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