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Agrophysical Research Institute: interdisciplinary and multidisciplinary studies for the practice of agriculture and plant production (1932-2022)

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90 YEARS OF AGROPHYSICAL INSTITUTE AS A HISTORY OF PRIORITY ACHIEVEMENTS IN RUSSIAN AND WORLD AGROPHYSICAL SCIENCE

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Abstract

The article described the main stages of the formation of the Agrophysical Research Institute over 90 years of its existence. Since its foundation in 1932 as part of the Lenin All-Russian Academy of Agricultural Sciences (VASKHNIL) at the initiative of Abram F. Ioffe and Nikolai I. Vavilov, the Agrophysical Institute focuses on establishing mechanisms of genotype—abiotic environment interaction in order to control the production process in agricultural plants both in the field and under controlled growing conditions. Accepting the ideas of N.I. Vavilov, A.F. Ioffe put forward the concept that the agrophysical science, relying on the achievements of physics, mathematics and biology, will ensure the transition from descriptive agronomy to agronomy based on measurements and calculations of factors of productivity, growth and development of plants and crops. This allows agricultural practitioners to manage crop formation and productivity. It is emphasized that the main objectives still are understanding the fundamentals of the functioning of agroecological systems and the development of scientific foundations, methods and means to research physical, physicochemical, biological and biophysical processes in soil—plant—active layer of the atmosphere. The research studies also aim at simulation mathematical models of these processes. The development of theoretical foundations, methods and tools for managing the productivity of agroecological systems for effective and sustainable agriculture and crop production in natural and regulated conditions are relevant. The development and creation of technical means of obtaining information about the state of plants and their habitats also are in focus. Nowadays, the Agrophysical Institute, as a leading research institute, implements scientific and technical programs and projects based on agronomic physics and related sciences, e.g., agroecology, soil science, genetics, biophysics and plant physiology, agroclimatology, computer science and computational mathematics, cybernetics and instrumentation. The Agrophysical Institute successfully develops new areas of research focused on the methods for effective management of the growth, development and productivity of crops through physical, physicochemical and other abiotic factors affecting the habitat of plants in order to modernize and intensify agriculture and the entire agro-industrial complex.

Keywords: agrophysics, development history, soil physics, soil science, precision farming, plant growth and development factors, crop productivity management

Agrophysical Research Institute (AFI) was founded in 1932 as part of the Lenin Academy of Agricultural Sciences of the USSR (VASKhNIL) on the initiative of academicians Abram F. Ioffe and Nikolai I. Vavilov for the development of a new branch of natural and agronomic sciences in those years, the agronomic physics. Its tasks included establishing the mechanisms of interaction between the genotype and abiotic environmental factors in order to control the production process of agricultural plants in field and controlled growing conditions.

In the scientific heritageof N.I. Vavilov's genotype-environment interaction took a central place in the formation of phenotypic and genotypic variability of economically valuable traits and formed the basis of the classical law of homological series in hereditary variability [1], the main value of which lies in its prognostic essence.

N.I. Vavilov repeatedly pointed out the presence of varietal and genotypic differences in agricultural plants in terms of ecological plasticity, as well as the need to study the nature of these differences and manage them with the help of abiotic environmental factors, primarily physical and physicochemical ones [2, 3]. That is why, as the President of VASKhNIL, he invited the physicist and outstanding organizer of science, Academician A.F. Ioffe (1880-1960), who, being director of the Physico-Technical Institute (PTI) of the USSR Academy of Sciences in 1930-1931, initiated agrophysical research at the PTI. This period coincided with the formation of institutions in the VASKhNIL system. In 1932 A.F. Ioffe created an institute that solves the urgent problem for agriculture of increasing crop yields by managing ecological-genetic and physiological-ecological processes of crop formation. Based on the ideas of N.I. Vavilov, A.F. Ioffe put forward the concept that agronomic physics (agrophysics), based on the achievements of physics, mathematics and biology, should ensure the transition from descriptive agronomy to a science based on measurements and calculations about abiotic factors that determine the productivity, growth and development of plants, and to the development of agricultural practices to control the production process and the formation of crops. For many decades, A.F. Ioffe improved the branch of agronomic science he created, which turned into an independent scientific direction.

Agrophysics studies the physical, physicochemical and biophysical processes in the system soil-plant-active layer of the atmosphere and the main laws of the production process, the physical characteristics of the components of the system and agricultural products. Scientific foundations, methods, technical and mathematical tools and agricultural practices are being developed for the rational use of natural resources, increasing the sustainability of agroecosystems, agriculture and crop production. This is a section of interdisciplinary agricultural science that studies the physical components and structural and functional patterns of their interaction, as well as the interaction of the genotype and the environment, the varietal diversity of crops in relation to agrocenoses and agrolandscapes with the aim of agroecological optimization of modern farming systems.

The results of agrophysical research are used not only in plant growing and agriculture, but also in other fundamental and applied areas, for example, in animal husbandry (by regulating the temperature in the barn, you can control the milk productivity of animals), microbiology (abiotic factors significantly affect the processes of interaction of soil microflora with the rhizosphere plants). Therefore, terminologically, the formulation "genotype-environment interaction" most fully reflects the subject of agrophysics as a branch of modern agricultural science.

The stages of the formation of this new field of knowledge, the priority achievements of the domestic agrophysical school, the prospects for the development of agrophysical science and the practical use of research results as the basis of high-tech agricultural technologies are associated with the history of the Agrophysical Institute and a galaxy of outstanding domestic scientists, the physicists and agrophysicists G.M. Frank, V.P. Malchevsky, S.V. Nerpina, F.E. Kolyaseva, B.S. Moshkova, N.F. Bondarenko, A.M. Globus, I.B. Revuta, A.F. Chudnovsky, I.B. Uskova, A.V. Kurtener, V.A. Semenov, V.P. Yakusheva, R.A. Poluektov and many others who made an invaluable contribution to the creation and development of the Agrophysical Institute.

Agrophysical Institute: creation and formation. The specifics of the territorial development of the quarter to the south of the Akademicheskaya metro station in the north-east of Leningrad (now St. Petersburg) on the left side of Nauki Avenue is to create a multidisciplinary educational and scientific complex, similar to Oxford (Oxford, UK) and California (Los Angeles, USA). Scientific and educational organizations are concentrated in this area. In the center of the complex is the Peter the Great St. Petersburg Polytechnic University around which the Ioffe Physico-Technical Institute RAS (PTI), Agrophysical (formerly Physical and Agronomic) Institute, Vedeneev All-Russian Research Institute of Hydraulic Engineering, AO Research Institute of Precision Mechanics, Central Research and Development Institute of Robotics and Technical Cybernetics, Institute of Direct Current, Institute of Cytology RAS, Voeikov Main Geophysical Observatory are located. These research institutes and educational institutions are interconnected in many ways. Joint developments and projects are carried out, scientific seminars, conferences and symposiums are held. Branches of university departments function at the institutes, students do internships and complete their theses in the laboratories of the institutes, graduates continue their studies in postgraduate studies at research institutes. Unique research stands become an inter-institutional experimental base.

Most of the research institutes were created in the 1930s on the initiative of the leading professors of the St. Petersburg Polytechnic University and with their direct participation. The Agrophysical Research Institute is the clearest example of such a link in scientific cooperation. A.F. Ioffe, the Director of the Physico-Technical Institute, who was also the dean of the Faculty of Physics and Mechanics of the Leningrad Polytechnic Institute, with the support of N.I. Vavilov took the initiative to create such an institute in the VASKhNIL system.

As a result, by the decision of the Board of the People's Commissariat of Agriculture of the USSR of January 5, 1932 and the protocol of the meeting of the All-Union Agricultural Academy of Agricultural Sciences Presidium of January 7, 1932, the Physico-Agronomic Research Institute (now AFI) was founded as part of the All-Union Agricultural Academy of Agricultural Sciences. The AFI was created using the technological base of the Physico-Technical Institute; employees of the Physico-Technical Institute and the Polytechnic Institute were involved in the work.

A.F. Ioffe formulated the initial theme of the institute's work, which corresponded to the problems that were relevant at that time. In the fundamental works of A.F. Ioffe [4-6] identified the main areas of research in this new branch of the natural sciences.

Agronomic physics as a science began to form at the end of the 18th century. In Russia, its founders were prominent agronomists, soil scientists and climatologists. The beginnings of the foundations of modern agrophysics were laid in the works of V.V. Dokuchaev, K.A. Timiryazev, P.A. Kostychev, A.A. Izmailsky, V.G. Rotmistrov, V.R. Williams, A.I. Voeikov and N.I. Vavilov. The term “agrophysics” was proposed by A.G. Doyarenko in relation to soil research. These scientists first drew attention to the importance of physical factors in plant life and formulated their comprehensive study as the main task of agronomic physics. Agrophysical research of that period was limited to field observations without rigorous physical experiment and mathematical analysis. Therefore, despite the outstanding achievements of the researchers of that time, they rarely managed to create complete, convincingly proven theories.

In 1930-1931 A.F. Ioffe at the suggestion of N.I. Vavilova took up the organization of agrophysical research at the Physicotechnical Institute of the USSR Academy of Sciences. After the establishment of the Institute of Physics and Agronomy, it was the employees of the Physicotechnical Institute who formed the core of the team of agrophysicists, who determined the main strategic scientific direction of their research as the control of the production process of plants based on measurements and calculations.

In subsequent years, a specific research methodology for agrophysics was developed. Firstly, it was focused on the control of biological processes at all stages of ontogeny through physical, biophysical, physicochemical effects directly on the control object and through the environment surrounding this object. Secondly, the basis of management decisions was the measurement of the parameters of the state of the system soil–plant–active layer of the atmosphere and its monitoring. Thirdly, the study of the processes of energy and mass transfer had to be carried out in the field and laboratory conditions on full-scale, laboratory physical and mathematical models. The institute has developed a specific approach to the creation of devices, which has become a distinctive feature of agrophysical instrumentation. Devices were developed from a laboratory sample that provides maximum completeness and accuracy of measurements of the physical process parameters but has low operational reliability to a serial device with an accuracy sufficient for managing this process in the field by technological personnel without special professional training.

Agrophysics, as a natural agricultural science, developed according to its own internal laws, however, each period of personal leadership of the scientific team of the institute brought new directions in addition to the main ones that were formulated by A.F. Ioffe and turned out to be so fundamental that all subsequent leaders steadily developed them.

API leaders. Doctor of Physical Sciences, Academician of the USSR Academy of Sciences A.F. Ioffe, as the first director of the institute and its scientific adviser, developed the methodology of the electronic agronomist between 1932 and 1960. Thanks to it, specific agrophysical instrumentation was developed, the use of semiconductors in agricultural instrumentation was created, information and measurement systems for obtaining, collecting and storing information were created, electronic computing tools were used to develop agronomic solutions when managing crop growing processes.

Doctor of Technical Sciences, Corresponding Member of VASKhNIL S.V. Nerpin, who led the AFI team from 1961 to 1975, focused research topics on the development of mathematical modeling methodology in the description, analysis and research of the production process of plants and energy and mass transfer in their habitat.

Under the leadership of Doctor of Technical Sciences, Academician of the Russian Academy of Agricultural Sciences N.F. Bondarenko, who held the position of director since 1975, the methodology of programmed harvesting using mathematical models of the production process of the main field crops, as well as mathematical statistics and probabilistic methods were developed. Research on this topic was carried out comprehensively by many laboratories of the institute with broad international cooperative participation (Bulgaria, Poland, Hungary, Czechoslovakia, and Germany). At the initiative of N.F. Bondarenko, the Special Design Bureau of the institute designed and created a system of instrumentation for information support of crop programming technologies. The yield programming methodology has been adopted by domestic and foreign agriculture and crop production.

In 1979-1996, the Institute was headed by Doctor of Physical and Mathematical Sciences, Corresponding Member of the Russian Academy of Sciences I.B. Uskov, who laid the foundations for new directions in agrophysics: the creation of a theory, methods and means of controlling the microclimate of fields and greenhouse complexes; development of agro-climatic research using probabilistic approaches and methods of mathematical statistics; development of theoretical foundations for wind erosion of soils and methods for calculating soil protection anti-erosion systems and agrotechnical measures; creation of methodology, principles and rules for applying the theory of similarity in agrophysics. In the 1970s, the institute intensified research on the adaptation of agriculture to observed and predicted weather instability.

Since 1996, the position of director of the AFI has been held by Doctor of Agricultural Sciences, Academician of the Russian Academy of Sciences V.P. Yakushev. Under him, research was intensified on the creation of computerized knowledge bases accumulated by agronomy, crop production, agrochemistry, agrophysics, agrobiology, agroclimatology, and necessary for the creation of automated complexes for the synthesis of agricultural technologies. Agrophysical adapters began to be developed for information support of crop growing technologies, carried out at the planned and predictive-operational time levels. Research has been launched on the scientific support of the principles for the implementation of innovative agricultural technologies of precision farming (PF), which has become the next stage in the development of the adaptive farming method, work to improve which continues at the institute.

For the first time in the world, the idea of precision farming was put forward by Academician A.F. Ioffe in the methodology of "electronic agronomist" developed by him. To implement it, A.F. Ioffe proposed to consider the community of plants in a field or in a greenhouse, their habitat and purposeful human activity as a single agroecological system that can be described in the language of mathematics and create conditions for choosing optimal agrotechnical solutions by analyzing quantitative estimates of the system's behavior under various conditions. With this approach, it becomes possible to predict the size of the crop that can be obtained in specific soil and climatic conditions on a given plot of land, evaluate the resources required for this, select ways to effectively manage crop formation, and also determine what changes the soil will undergo as a result of applying that or other technology.

This research ensured the transition from experimental and descriptive agricultural science to the identification of quantitative patterns and theoretical generalizations. The prerequisites were founded for the emergence of a qualitatively new methodology for managing agricultural technologies. Its main feature is the transition from intuitive decisions based only on the experience of the farmer to quantitatively based methods of managing technological processes using computers. This led to the emergence of a new scientific and practical direction — harvest programming.

The ongoing research on PF is largely a logical continuation of work on crop programming [7, 8]. The theory of PF can be considered as a natural continuation of the development of the theory of crop programming at a new stage of scientific and technological progress using information technologies, global navigation systems (GLONASS/GPS), geographic information systems (GIS) and the Internet. The fundamental difference between PF and crop programming is that the recommended solution obtained using computer calculations is automatically implemented taking into account intra-field differentiation in a given agricultural field. When programming yields, the final decision remained with the agronomist, and the differentiation of the norms of technological impact could be carried out only from field to field.

Since 2016, the Agrophysical Institute has been headed by Doctor of Biological Sciences, Corresponding Member of the Russian Academy of Sciences Yu.V. Chesnokov. He took an orientation towards the integration of systems of precision farming, soil science, agrometeorology and land reclamation with adaptive crop production. In fact, this integrative process was identified in the fundamental works of Academicians A.F. Ioffe and N.I. Vavilov and is the establishment and use of the mechanisms of genotype-environment interaction to control the production process of plants in modern conditions and at a new scientific and technological level.

The research currently being carried out at the AFI can be divided into two main areas. The first is the improvement of plant growing technologies to optimize the production process (such as the technology of differentiated application of fertilizers, ameliorants and other agrochemicals), including the assessment of the condition of plants and crops by remote methods. The second direction is the improvement of plants using innovative breeding and agrophysical technologies, as well as obtaining varieties that are superior to the existing crops in terms of yield and quality, resistant to soil and climatic conditions of cultivation regions, efficiently using natural resources (light, water, elements of mineral nutrition, etc.), responsive to the application of fertilizers, ameliorants and other agrochemicals. Within these areas, methods are also being developed based on digital non-invasive optical, x-ray and other agrophysical methods for monitoring the physiological state of plants and crops, as well as their accelerated phenotyping according to economically valuable traits, productivity and stress resistance to abiotic environmental factors. In addition, reclamation and soil-agricultural theories have been developed (especially in the field of physics, physical chemistry and biophysics of soils, including their water, gas regime and physical and mechanical properties), the theory and practice of chemical reclamation of acidic soils; research is being conducted on agroecological monitoring and finding ways to reduce the accumulation of toxic substances and elements in agricultural products; the scientific foundations of systems for the reproduction of soil fertility, the use of fertilizers, crop rotations, and tillage are being developed; research is being carried out on the creation of working bodies of drainage-flushing machines for closed tubular drainage and the preparation of regulatory documents for field reclamation and other areas of research work.

Further development of scientific areas. Research by Professor F.E. Kolyasev (1898-1958) contributed greatly to the beginning of the development of agricultural theory in agrophysics. In his works [9, 10] the techniques and methods for managing the water balance of soils in various soil-climatic zones of the country are considered, and the theory of differential soil moisture is developed.

The works of Professor P.V. Vershinin (1909-1978) [11, 12], which covered the theoretical and practical issues of soil structuring, the use of chemical structure formers, including for protection against erosion, were important in creating the theory and methods of soil structure management. Academician E.I. Ermakov (1929-2006) made a significant contribution to the development of the theoretical foundations of the soil-forming process in the system of plant-soil complex. Thanks to the scientific school of Professor I.B. Revut (1909-1978) laid the agro-ecological foundations of soil cultivation for the soil-climatic zones of the country, and soil physics was introduced into agriculture [13-15].

Corresponding Member of VASKhNIL S.V. Nerpin (1915-1993) created two directions in agrophysical soil science: soil hydromechanics and theoretical physicochemical soil mechanics. His monographs [16, 17], in which the theory and methods of field water regime management are developed, have no analogues in the world scientific literature. The fundamental works of Professor A.M. Globus (1930-2008) [18, 19] actually laid the foundation for research in soil hydrophysics.

Professor M.K. Melnikova (1901-1986), head of the first radiochemical laboratory in Russia, was the initiator of extensive research on the physical chemistry of soils and the founder of the study of the behavior of uranium and plutonium fission products in soil. In her studies [20, 21] the method of isotope tracers in soil science was developed for the first time. Prof. Yu.A. Kokotov made a notable contributions to the theory of ion exchange in soils, including soil acidity, [22-24]. Professor N.F. Batygin (1928-2000) created the theoretical basis for the agrophysical trend in Russian radiobiology [25].

In 1928, a sector of agroclimatology was organized at the State Institute of Experimental Agronomy. In 1932, research on agro- and microclimatology for agro-climatic zoning and assessment of climatic resources in agricultural production was launched at the Voeikov Main Geophysical Observatory, but in August 1935 this work was stopped. At that time, simiar investigations began in the AFI, where research was focused on the analysis of the physical processes that form the microclimate of fields and crops, in contrast to the geographical climatic approach adopted in the Voeikov Main Geophysical Observatory.

Professor A.F. Chudnovsky (1910-1985) [26-28], an associate and follower of A.F. Ioffe, in the monographs [26-28] developed a theory and methods for controlling the thermal regime of soils. Already the first issue of scientific works of the Agrophysical Institute in 1935, included articles on the thermal and water regimes of soils by B.P. Aleksandrov, A.V. Kurtener, and N.N. Banasevich. The third issue published in 1941, contained a section entitled “Microclimate Issues”". The monographs of professors D.A. Kurtener and I.B. Uskov were devoted to the development of the theory of agroclimatology and its practical aspects [29, 30]).

The beginning of research in the field of ecological physiology of plants was the world-famous monograph “Physiological basis of drought resistance of plants” [31] by one of the first plant physiologists of the API, Academician N.A. Maksimov (1880-1952). Professor V.P. Malchevsky (1906-1942) developed methods of light culture and light stimulation in greenhouses and indoors with artificial light sources, he proposed light stimulation of seeds of seedlings to increase the intensity of photosynthesis and reduce the growing season. Works by V.P. Malchevsky made a significant contribution to the creation of the foundations of the theory of light physiology and light culture of plants.

The term “biocybernetics of plants” and the ideology of this approach were proposed by V.G. Karmanov (1913-1997) and developed as a science of cybernetic control of physiological processes in a plant. Phytomonitoring (S.S. Radchenko) and biophysical ideas about the processes of transport and energy exchange in a plant, including cell membranes (Professor O.O. Lyalin, 1932-1994) became a key element of biocybernetics. Based on the developments of V.G. Karmanov, the first vegetative lighting installation was created, after which new areas of research in light physiology began to develop at the institute and a series of domestic artificial climate vegetative installations was created. Proceedings [32-34] of the corresponding member of VASKhNIL B.S. Moshkov (1904-1997) gained worldwide fame and made a fundamental contribution to the theory of light physiology. They discovered the physiological role of the leaf as an organ that receives photoperiodic exposure. The phenomenon of photoperiodism made it possible to explain some regularities in the distribution of cultivated plants discovered by N.I. Vavilov. On the basis of photoperiodism, techniques have been developed to control the growth and development of plants. B.S. Moshkov showed in lighting installations the possibility of using light-periodism for pseudo-control of mutagenesis and the selection process under controlled conditions. On an in-depth genetic basis, research has been expanded to improve the efficiency of selection using the advantages that such an approach provides. The technology of growing plants in protected ground under electric lighting has been developed.

The methodology for creating perfect regulated large-scale agroecosystems, proposed by Academician of the Russian Academy of Agricultural Sciences E.I. Ermakov (1929-2006), based on the principles of physical modeling proposed by him. Such agroecosystems ensured the year-round production of high-quality plant products with a given biochemical composition [35]. Under the leadership of E.I. Ermakov, original vegetation and irradiation equipment, including a unique rhizotron technique for a two-dimensional root sphere were designed. On the basis of regulated agroecosystems, comprehensive studies of the evolutionary transformation of initially abiogenic substrates into soil-like bio-inert formations were carried out. The bases and methods of bioremediation of soils contaminated with liquid propellant components, oil and oil products have been developed. The genetic research on the programming of transgressions for plant breeding and introduction has been further developed. Works have begun, among other things, to study the water status of plants, their response to the action of increased doses of ultraviolet radiation and other abiotic stress factors of a physical and physico-chemical nature.

The Special Design Bureau of the Agrophysical Institute (Yu.P. Baryshnev) has developed and put into production a thematic series of original vegetative-climatic installations with controlled temperature, light and humidity conditions in a wide range of parameters. In 1982, these developments were awarded the State Prize of the USSR. For a long time, domestic phytotrons, physiological and selection-genetic laboratories were equipped with such installations.

The international expert community has recognized AFI as a leading scientific school that determines the formation of agrophysics as an independent branch of the natural sciences. Scientific organizations of a similar orientation were created in Bulgaria, Australia, Hungary, Switzerland and Germany. The famous Polish physicist Professor B. Dobzhansky (Bohdan Dobrzański, 1909-1987), after visiting the Leningrad Institute, achieved the creation in Lublin of the world-famous Institute of Agrophysics as part of the Polish Academy of Sciences.

An important stimulus for the creative cooperation of scientists was the publication of scientific papers of the API. The first issue was published in 1935 and was devoted to the problems of soil structure and the thermal regime of soils. Subsequent thematic collections were published annually (from one to five issues) until 1998.

The release of the international scientific journal on agronomic physics "International Agrophysics" was organized thanks to the community of scientists from Europe, Asia and America. The journal was published at the Department of Physics of the University of Agricultural Sciences (Budapest) and was first devoted to the study of the physical properties of soils and the quality of agricultural products. Since 1985, the publication of the journal was taken over by the Institute of Agrophysics of the Polish Academy of Sciences with the expansion of the scientific fields represented (physical aspects of the study of the environment and agricultural sciences).

Modern directions of scientific research in API. Proceedings of Academician N.F. Bondarenko (1928-2003) ensured the development of agrophysical scientific support and expanded practical application of the crop programming method. Based on these, information technologies for precision farming were developed [8].

With the development of branches of physics, geophysics, biophysics and plant physiology, mathematical physics and computational mathematics related to agrophysics, new areas of agrophysical research arose, focused on the development of methods of active intervention in the processes of growth and development of crops and management of these processes. These areas [36-40] include mathematical simulation of the production process of agricultural plants (R.A. Poluektov); development of the theory of similarity of agrophysical systems and processes (B.N. Michurin, A.M. Globus, I.B. Uskov, V.G. Onishchenko); analysis of the interaction of biological objects with physical fields of various nature — light, gravitational, magnetic, electromagnetic, acoustic, electrostatic (B.S. Moshkov, N.F. Batygin, N.F. Bondarenko, I.S. Lisker, V.N. Lazutin); creation and optimization of informatic technologies for managing the production process in agriculture and crop production (V.P. Yakushev, I.M. Mikhailenko); design and instrumentation of vegetation plants with controlled climate (V.G. Karmanov, A.F. Chudnovsky, Yu.P. Baryshnev); agrophysical instrumentation (I.P. Ananiev, Yu.I. Blokhin); development of coordinate precision farming as a modern continuation of the methodology of the “electronic agronomist” (V.P. Yakushev).

Climate change is becoming an increasingly important topic on the global agenda every year. In 2021, the management of carbon dioxide emissions was one of the most discussed issues in the world’s highest ranking forums. Climate change forecasts from the world's leading institutions, obtained using many different models, are based on different options for the expected amount of CO_2 emissions. Such forecasts are systematized and evaluated by the Intergovernmental Panel on Climate Change (IPCC, Switzerland). The AFI Agroclimate Laboratory has proposed original methods for collecting and analyzing such forecasts, which, in combination with already available agrometeorological data, allows obtaining impartial estimates of probable climate changes based on fuzzy logic mathematics, easily adaptable for machine learning and analysis using artificial intelligence.

The observed climatic changes are accompanied by an increase in the frequency of occurrence of phenomena dangerous for crop production, which requires solving the problem of managing agro-climatic risks. AFI has developed software that allows processing large amounts of data on the existing and predicted agroclimate in combination with the measured agrochemical parameters of the soil and, on this basis, to make probabilistic forecasts of yields of the main crops that can be mapped for a specific user, taking into account the microclimate of the fields [41-45].

Precision farming (PF) is one of the areas of modern research work at the API, developed since 2002 under the guidance of Academician V.P. Yakushev [8], without exaggeration, can be called the world trend of adaptation of crop production to intra-field variability of crop formation conditions. Reasonable planning and subsequent differentiation of technological impacts in modern farming systems are directly dependent on the degree of intra-field heterogeneity [7]. The creation of reliable and accessible methods for detecting such heterogeneity, determining the degree of its intensity and spatial distribution in agricultural fields is a key task in managing crop production in the PF system.

A promising scalable resource for solving this problem is Earth remote sensing (ERS) data [8], the interpretation of which makes it possible to carry out a continuous continuous assessment of the state of crops and their habitat while simultaneously covering large areas. In Russia, with its large territory (and with the increasing availability of aerospace data), there is no alternative to such an approach in the information support of modern agriculture. Aerospace remote sensing data, ground-based measuring systems and mathematical models make it possible to significantly improve the quality and scale of information support for agriculture, monitoring of large natural objects and phenomena (land cadastres, forests, water bodies, fires, floods) [46].

AFI scientists led by V.P. Yakushev proposed two new methods of using remote sensing. The first involves the use of variogram analysis of satellite images, the second is based on a comprehensive assessment of the dynamics of changes in the optical indicators of reflection indices calculated from hyperspectral images. A basic algorithm has been developed for detecting and highlighting the boundaries of intra-field heterogeneity using hyperspectral images of agricultural fields and using optical criteria (reflection indices) that characterize specific and non-specific features of the spectral indicators of sowing under the influence of stressors. To implement the geostatistical approach, a toolkit was created and tested for constructing empirical variograms and their approximations based on remote sensing data, which functionally describe the statistical structure of spatially varying indicators of the state of the soil or sowing on an agricultural field. The prospect of automating this process has been studied. Semivariogram analysis is an effective method for characterizing the structure of data spatial variability. It is widely used to evaluate the spatial heterogeneity of surface reflectance values and improve image classification. It should be noted that the use of new methods of analysis and interpretation of satellite data would significantly increase the scale of information support for PF technologies.

In 2009-2017, the concept and theoretical basis for the management of agricultural technologies in precision agriculture was developed, which continues to be improved [47]. According to the proposed concept, the general task of managing agricultural technology includes four levels of tasks that are solved on different time scales. At the upper, 1st level, the problem of managing crop rotations on an annual scale is solved; on the 2nd, implemented on a daily scale at one vegetation interval, the task of program control; tasks of the 3rd and 4th levels are implemented in real time. At all levels, the object of management is the field with the sowing of agricultural crops. However, the concept does not take into account the fact that the agrocenosis, in addition to the main crop, includes annual and perennial weeds. They compete with the crop for moisture and nutrients, and crop losses from weed infestation can exceed 50%. Therefore, optimal technological programs throughout the growing season should include not only fertilization and irrigation operations, but also herbicide treatments. Such programs should be formed taking into account the fact that mineral nutrition stimulates the growth of both cultivated plants and weeds, and herbicides not only suppress the growth and development of weeds, but also act depressingly on cultivated plants.

The formation of a unified management program that takes into account the state of the main crop and weeds and includes the simultaneous application of mineral fertilizers and herbicide treatment makes it possible to avoid crop losses and excessive consumption of fertilizers. In addition, optimization of fertilizer doses that meet the biological needs of the crop in nutrients activates metabolic processes, accelerates herbicide inactivation and increases resistance to it. That is, due to a more intensive accumulation of organic mass, a growth decrease in the concentration of the herbicide in plant tissues occurs, and smaller amounts of the drug are inactivated faster with optimal metabolism. Optimal nutritional conditions also increase the overall biological competitiveness of the crop in relation to weeds. Therefore, the combined use of herbicides with mineral fertilizers is one of the effective methods of weed control in crops and a significant increase in crop yields. To implement such an idea, a significant refinement of the concept and theory of management of agricultural technologies was required: new mathematical models of agrocenosis, optimality criteria and algorithms for the formation of control programs were proposed. Fundamentally different robotic technological machines will also be needed, which will significantly speed up the process of crop management.

The currently observed extreme manifestations of weather conditions can lead to an imbalance in such ecological functions of the soil—plant—active layer of the atmosphere system as biogeochemical circulation, energy and moisture exchange, accumulation, transport and removal of nutrients, buffer capacity. There are uncertainties in accurately assessing the conditions of sustainability (ability to resist impacts) and recovery (speed and degree of return to the original dynamic equilibrium) of the balance of these ecological functions of the specified system. Therefore, a holistic analysis of the state of the system before and after impacts should be based on the results of interdisciplinary related studies (48).

Improving the methodology for studying the stability and restoration of the soil—plant—active layer of the atmosphere system after natural and anthropogenic impacts in the API is carried out in three related interdisciplinary areas.

As part of the first direction, instrumental studies of physical (density, aggregate composition), physicochemical (pH), hydrophysical (moisture content, main hydrophysical characteristic, moisture conductivity) and, in the future, thermophysical (temperature, thermal conductivity, heat capacity, thermal diffusivity) properties of soils are carried out [49-51]. The results obtained are necessary for analyzing the close relationship between soil properties in assessing their stability and recovery after natural and anthropogenic impacts.

The second direction includes instrumental analysis of carbon and nitrogen cycles, which are fundamental interdependent processes in the global biogeochemical cycle of substances in ecosystems. Modern studies are aimed at conjugated assessment of the degree of interrelationships between carbon and nitrogen cycles in various climatic conditions with the dynamics of temperature, moisture and oxygen content, available nitrogen and carbon compounds, microbiological and enzymatic activity, mainly in the uppermost part of the soil genetic profile. First, instrumental studies generally involve an analysis of the influence of natural and anthropogenic impacts on the sequestration of organic matter in soils and their clay fractions [52, 53]. The intensity of sequestration and the degree of carbon fixation in the genetic profiles of soils are largely determined by the amount and mineralogical composition of their clay fraction, the content of iron hydroxide under various redox, hydrophysical, thermophysical, biochemical, and microbiological conditions. That is why, in order to improve the methodology, it is necessary to deepen knowledge about the fundamental mechanisms of carbon sequestration in soils as a result of interactions of nonspecific (carbohydrates, lignin, lipids, phenols, amino acids) and specific (humic acids) organic compounds with primary and secondary clay minerals in soil genetic profiles in order to assessment of the significance of these mechanisms in maintaining sustainability and restoring the balance of the carbon cycle. Secondly, the modern study of carbon and nitrogen cycles in various land use systems is mainly devoted to the analysis of the influence of soil properties on the microbiological processes of the formation of carbon dioxide (CO_2) and nitrous oxide (N_2O) in them, as well as on the intrasoil and direct fluxes of these substances from soils [54-56]. A quantitative assessment is required of the conditions for the formation of dominant processes of organic matter mineralization, autotrophic and heterotrophic respiration, nitrification and denitrification in the profiles of various soils, the predominant ways of transport of CO_2 and N_2O in soil profiles, the contribution of intraprofile transport of CO_2 and N_2O to their direct emissions from soils, as well as the role the aforementioned managed processes in maintaining sustainability and restoring the balance of carbon and nitrogen cycles.

Within the framework of the third line of research, an analysis is made of the closeness of the relationships between the meteorological parameters of the surface layer of the atmosphere, as well as the components of the heat and water balance of the underlying surface, and the hydrophysical and thermophysical properties of soils (57-59). Improving the methodology involves a more correct description of latent and explicit heat and moisture fluxes between the stratified ground air layer and a rough underlying surface, a deeper analysis of the mechanisms of transport of heat fluxes, capillary and film moisture in the genetic horizons of soils, a reasonable analysis of the degree of relationship between heat and moisture fluxes in soil and from the underlying surface in order to accurately assess their conjugated contribution to achieving the required stability conditions and restoring the soil—plant—active layer interaction.

The Agrophysical Institute continues research on topical issues of field experiment methodology, agroecological monitoring, management of effective soil fertility, phytosanitary condition and productivity of agrocenoses. For example, a multilevel system of field experiments has been created in the Menkovsky branch of the AFI, which serves as a methodological basis for large-scale fundamental and applied research [60]. Thanks to it, using new methodological approaches, the fundamental and applied foundations of precise crop fertilization systems [61, 62], the methodology of phytosanitary monitoring and applied aspects of precision integrated plant protection [63, 64] were significantly developed, theoretical aspects of the interaction of ameliorants with soil were developed, and adaptation problems were studied. , agro-ecological and agro-economic aspects of melioration and cultivation of soddy-podzolic soils, including those on re-developed agricultural lands [60, 62, 65].

Special attention in the API is given to land reclamation as one of the ways to adapt Russian agricultural production to climate change, which can significantly affect the agrophysics and physical chemistry of soils, in particular, their water permeability. Thus, the increase in temperature in winter significantly reduced the depth of soil freezing in the Nonchernozem zone of Russia (on average from 100-120 to 45-70 cm) and, as a result, limited the cryogenic restoration of the vertical pore space, which plays an important role in the formation of the total water permeability of the soil stratum [66]. This fact had a significant impact on the efficiency of closed drainage systems. Its negative consequences are observed when drains are drained from fields drained by closed tubular drainage during heavy rainfall. In this regard, in recent years, the Agrophysical Institute has begun studying the restoring of closed tubular drainage with an expired service life and siltation of more than 80% of the tubular drain cross section. Theoretical studies of the processes of destruction and transportation of silt deposits in the tubular cavity of the drain to its mouth, tested on models and on a specially designed research stand, made it possible to establish the manufacture of milling working bodies of drainage flushing machines intended for operation during the restoration of a faulty drainage tubular drainage [67]. In addition, scientific and practical guidance has been issued to reduce the risks of growing crops on drained reclaimed land in a changing climate [68)]. Comprehensive tests have been carried out and regulations for the production, use and technical conditions for more than two dozen ameliorants and fertilizer materials have been developed. In Russia, liming of more than 70% of acidic soils is carried out using the regulatory and technical documentation developed at the Agrophysical Institute.

Using remote sensing methods, reclaimed lands were surveyed and the technical condition of drainage systems was determined. The developments of the Agrophysical Institute on the assessment of agricultural melioration facilities using unmanned aerial vehicles have found application in the design, repair and construction of such facilities (69).

Modern research on plant light physiology and bioproductivity of agroecosystems is focused on understanding the patterns of interaction between plants and associated biota and habitat in a regulated agroecosystem when modeling optimal and stressful conditions, as well as on developing methods and means to increase plant resistance to stress factors and obtain stable high yields of the required quality in conditions of protected and open ground [70, 71].

Particular attention is paid to the genetic breeding methodology for increasing the productivity and resistance of agricultural crops to the ecological-geographical and landscape-climatic conditions of the growing regions, obtaining new forms with a predictable set of economically valuable properties, taking into account the specifics of the genotype-environment interaction [72, 73].

For decades, the API has been developing highly efficient resource-saving phytobiotechnologies and original light-irradiating equipment for year-round intensive production of plant products with specified quality and functional characteristics [71, 74]. Systems for rapid assessment of the physiological state of vegetative plants and the quality of seed material were tested using information-measuring means of phytomonitoring, optical and radiographic methods [75-77].

Continued study of the mechanisms of genotype-environment interaction under controlled conditions will deepen our understanding of the fundamental principles of controlling the production process of plants and regulating the flow of biogenic elements in agroecosystems. These data will also be used to create plant forms that are highly valuable in terms of productivity and quality, obtained using the original genetic breeding methodology, next-generation bio-, nano-, agrotechnologies and applied digitalization [71, 78-80]. Among the immediate scientific and practical tasks, there are optimization of production process in intensive light culture and the creation of high-tech automated autonomous stationary and mobile phytotechnological complexes with original vegetation-and-irradiation equipment and resource-saving agricultural technologies for continuous year-round production of plant products with specified functional and quality characteristics in the immediate vicinity to the consumer, regardless of natural and climatic conditions [71, 81, 82]. Obtaining highly productive forms of plants (including marker-assisted selection, MAS) [83-85], intended for intensive light culture, is an essential element of the proposed original effective interdisciplinary methodology and technologies for the formation of plant products and raw materials with specified qualitative and quantitative characteristics [71, 86].

Another promising scientific direction is the practical use of microorganisms. In particular, microorganisms that simultaneously produce monooxygenase and hydrolytic cellulases are of interest for the conversion of plant residues and the regeneration of spent organomineral substrates [79, 87]. The API develops the fundamental principles for the participation of microorganisms in the generation of electricity in the plant-microorganisms-rhzosphere system [80, 88]. When creating biopreparations with a complex action, the peculiarities of the secretion of microbial exometabolites and their influence on the production process of agricultural crops under favorable and stressful conditions are taken into account [71].

The basis of integrative interdisciplinary studies of the genotype-environment interaction will be i) the digital methodology for monitoring the production process (including PF) [8, 89-91] and the quality of seed material; ii) diagnosing and assessing the state of fields using remote sensing methods and predicting crop productivity [46, 92, 93]; iii) determination of plant tolerance and adaptability to abiotic stressors using non-invasive optical, X-ray and other physical methods of analysis and digitalization [75-77].

International scientific cooperation. Many agrophysicists and organizers of science from the Baltic countries, Transcaucasia, Central Asia, China, Vietnam, Eastern and Western Europe began their scientific career at AFI. The Agrophysical Institute has strong ties with leading research centers and universities in Poland, Germany, Hungary, Moldova, Belarus, Slovakia, the Czech Republic and other countries.

Thus, in the framework of scientific cooperation with the Slovak Agricultural University (Slovenská poľnohospodárska univerzita — Slovak University of Agriculture, SUA, Nitra, Slovakia), Russian and Slovak scientists carried out joint studies of energy and mass transfer processes in the soil–plant–atmosphere system. It is shown that the use of biochar provides many advantages to agriculture by improving the whole range of soil properties, including its structure. However, various effects of biochar exposure depend on its physical and chemical properties, application rates, initial soil properties, etc. The field trials were carried out in 2017-2019 with Haplic Luvisol biochar at the SUA experimental station. Initial as well as repeated application of biochar resulted in improved soil structure. Increasing soil organic matter from initial and reintroduced biochar significantly maintained the stability of soil aggregates, while humic-based organic matter did not provide such stability [52, 57].

Scientific and technical cooperation is developing with TOO Baraev Scientific and Production Center for Grain Farming (Akmola region, Republic of Kazakhstan) on the effective use of software, hardware and technical systems in agricultural production. At a specialized experimental site in the Republic of Kazakhstan, the prospects for using the precision farming system developed at AFI were evaluated. On its basis, a new algorithm is proposed that uses remote sensing data and ground measurements, which allows you to move from inefficient monitoring activities and a static assessment of the observed phenomena to precision control methods and the prompt use of crop correction tools that increase the productivity of spring soft wheat. In addition, according to the dynamics of changes in agrolandscape field conditions determined by the terrain, it was possible to describe the distribution of watercourses that characterized the reserve of productive moisture in one or another part of the field [94, 95].

The Agrophysical Institute and the Center for the Study of Agricultural Landscapes (The Leibniz Center for Agricultural Landscape Research, ZALF, Müncheberg, Germany) conduct joint research on the creation and use of dynamic models of the production process of agricultural plants in applied and theoretical problems of agroecology. Thus, the API proposed an integrated system for modeling the production process of agricultural crops, which is applicable for the analysis of agricultural technologies, in particular, alternative strategies for planning crop rotations in various farming systems. It has been shown that the processes of flowering and seed maturation in plants, as a rule, are better modeled by the median of the ensemble of models than by the average of the ensemble and individual models. The yield is more accurately estimated not by an ensemble of models, but by the best models. Higher accuracy is usually achieved for spring crops, best results are obtained for maize for silage, and the lowest performance (in terms of agreement index) was noted for winter rapeseed. It has been established that only models with reasonable accuracy (i.e., without failures) for all crops in the target environment should be selected for crop rotation. In general, the use of the developed ensemble of crop models is one of the ways to improve the accuracy of forecasts, but relatively low variability of the output of the ensemble is possible, which indicates the variogram of the studied fields for different types of cultivated crops [96].

Since 2016, the Agrophysical Institute has been participating in the work of the ISTA technical committee (Advanced Technologies Committee, International Seed Testing Association, Wallisellen, Switzerland) on new technologies for assessing the quality of seed material. In 2022, at the 33rd ISTA International Congress (Cairo, Egypt), API presented a report "Software for processing and analysis of digital X-ray images of seeds" (https://www.seed-test.org/api /rm/R4G8AC7KS6QARNA/4-software-for-processing-and-analysis-of-digital.pdf), covering a number of API developments. These are software for improving the quality of initial digital X-ray images of seeds, VideoTesT-Morphology software for automatic analysis digital x-ray images of seeds, software Passport-Zerno for automatic recognition of the main types of hidden defects in seeds of grain crops, e.g., wheat, barley, rice, rye). Also, the Russian GOST R 59603-2021 “Crop Seeds. Methods of digital radiography” was announced. The presented inventions will further allow harmonizing the specified national standard with the current ISTA Rules in terms of X-ray analysis of seeds. Previously, approaches to seed radiography proposed in the API were reflected in publications in Russian and foreign scientific journals [97, 98].

The cooperation of scientists from the Agrophysical Institute with colleagues from the Leibniz-Institute for Plant Genetics and Crop Research (Leibniz-Institut für Pflanzengenetik und Kulturpflanzenforschung, Gathersleben, Germany) is one of the longest and largest. A number of studies have been carried out to establish the ecological and genetic mechanisms of genotype-environment interaction, in particular, to identify and identify genes and chromosome loci that determine economically valuable traits in spring common wheat. As a result, Russian and German partners have identified QTL (quantitative trait loci) which determine of more than 40 economically valuable traits in the conditions of the agroecobiological polygon [99]. The QTL of diffuse leaf reflectance indices have been mapped [75], and associative mapping and genome-wide study of chromosome loci and genes that determine the trait of frost resistance in spring common wheat (*Triticum aestivum* L.) were carried out [100-102]. In addition, in collaboration with colleagues from the Institute of Agricultural Microbiology (St. Petersburg-Pushkin, Russia) and the CSIR — National Botanical Research Institute (Lucknow, India), resistance to the highly toxic nickel metal was studied in T. aestivum in the interaction of plants with rhizobacteria and the stages of rhizobial defense mechanisms in plant-bacterial associations under nickel stress were determined [103]. It has been shown that both partners (plant and bacteria) are able to reduce nickel toxicity and have developed different mechanisms and strategies that manifest themselves in plant-bacterial associations. In addition to physical barriers such as plant cell walls, thick cuticles, and trichomes that reduce abnormal nickel intake, plants reduce nickel toxicity using their own antioxidant defense mechanisms, including enzymes and other antioxidants. Bacteria, in turn, effectively protect plants from nickel stress and can enhance phytoremediation [104, 105].

Thus, over the 90-year history, the Agrophysical Institute founded on the initiative of Academicians A.F. Ioffe and N.I. Vavilov has gained wide international recognition. The main research are focused on fundamental functions of agroecological systems, on development of scientific foundations, methods and means for studying physical, physico-chemical, biological and biophysical processes in the soil—plant—active layer of the atmosphere, on creation of simulation mathematical models of these processes. The development of theoretical foundations, methods and tools for managing the productivity of agroecological systems are carried out in order to increase the efficiency and sustainability of agriculture and crop production in natural and regulated conditions. Technical means are being developed to obtain information about the state of plants and their habitats. The research uses methodological approaches of agronomic physics and related sciences, i.e., agroecology, soil science, genetics, biophysics and plant physiology, agroclimatology, informatics, computational mathematics, cybernetics and instrumentation. Innovative research area that is successfully developing includes methods for effective management of crop growth, development and productivity through the influence of physical, physico-chemical and other abiotic factors on the plant habitat.

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BIOELECTROCHEMICAL SYSTEMS BASED ON THE ELECTROACTIVITY OF PLANTS AND MICROORGANISMS IN THE ROOT ENVIRONMENT
(review)

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Abstract

Bioelectrochemical systems (BES) based on electroactive processes in the root environment of plants and accompanying microorganisms are a new promising environmentally friendly technology for generating renewable energy. Although the possibility of practical use of bioenergy resources has already been shown in many studies, the nature of electrogenesis and the influence of external parameters on it have not been fully identified. The emergence of a potential difference in living systems is due to a complex of physicochemical processes that maintain an uneven distribution of ions at the cellular, tissue and organism levels (N. Higinbotham, 1970). In the process of plant development along the whole organism, a gradient of electrical potentials arises due to the diffusion of ions, concentration effects and differences in the intensities of biochemical processes (T.A. Tattar et al., 1976). Along with this, microorganisms of the rhizosphere are able to oxidize organic matter secreted by the roots (L. De Schamphelaire et al., 2010), while synthesizing carbon dioxide, protons and electrons. The ions and electrons formed in the course of redox reactions diffuse through the inhabited medium, leading to charge separation (B.E. Logan, 2008); as a result, a gradient of electropotentials is established, associated with differences in the concentrations of charged substances. A complex of processes for converting chemical energy from organic substances into electrical energy forms is the basis of the plant-microbial fuel cell (PMFC). The most common configuration of the PMFC device consists of an anode and cathode chambers, an ion-selective membrane (D.P. Strik et al., 2008); there are also various modifications in the form of a flat plate (M. Helder et al., 2013), a tubular configuration (R.A. Timmers et al., 2013), aimed at increasing the output electrical characteristics. One of the most important components of a BES are electrode systems. Most often carbon materials, which have high electrical conductivity, corrosion resistance, and a large specific surface area, are used. The productivity of BES depends on the composition of the root environment, the presence of potential-forming ions, and on the parameters of the light environment, the efficiency of photosynthesis. A promising option for using PMFC is their combination with significant production processes, in particular, their introduction into agricultural production. The possibility of using BES is shown on a number of cultivated and industrial plants with obtaining the following low-power energy output when growing rice — 140 mW/m^2 (N. Ueoka et al., 2016), lettuce — 54 mW/m^2 (T.E. Kuleshova et al., 2021), *Reed mannagrass* — 80 mW/m^2 (R.A. Timmers et al., 2012), *Common reed* — 42 mW/m^2 (J. Villasenor et al., 2013), cattail — 93 mW/m^2 (Y.L. Oon et al., 2016), *Common cordgrass* — 679 mW/m^2 (K. Wetser et al., 2015), etc., which have found application as food products, fuel, building materials, animal feed, etc. Prospects for the use of BES include power supply for environmental sensors (A. Schievano et al., 2017), light sources (W. Apollon et al., 2020), wireless sensor networks (E. Osorio-De-La-Rosa et al., 2021), the Internet of things (IoT) (Jayaraman P.P. et al., 2016),, phytomonitoring systems in natural conditions, greenhouses, remote areas, partial power supply of plant life support devices in artificial agroecosystems (T.E. Kuleshova et al., 2021), wastewater treatment (L. Kook et al., 2016).

Keywords: green energy, plant-microbial fuel cell, bioelectrogenesis, electroactive bacteria

Currently, the energy market is mostly occupied by fossil fuels — coal, oil and natural gas, the consumption of which leads to environmental pollution and climate change. Thereof, the use of environmentally friendly renewable natural energy resources is relevant. Solar energy, wind, geothermal heat, hydrothermal energy, biofuels are intensively used to generate electricity. However, they also have disadvantages, such as high installation costs, dependence on weather conditions and time of day, landscape transformation, and geographic localization. Against the background of these limitations, bioelectrochemical systems (BES) based on electroactive processes accompanying the vital activity of plants and surrounding rhizospheric microorganisms have the development potential.

The use of bioenergy resources for the development of a new field of "green" energy is a complex and not fully understood task that requires the integration of a wide range of knowledge in the fields of physics, electrochemistry and biology.

The purpose of this review is to analyze the existing designs of bioelectrochemical systems, describe the electrogenic and potential-forming reactions occurring in BES, and the influence of individual environmental factors on them, as well as consider the prospects for using bioenergetic devices.

Electrical processes in the root environment. *Plant electrogenesis.* The history of research on the electrophysiological properties of plants dates back more than a hundred years, but the mechanism of bioelectrogenesis, that is, the ability to move a charge and generate electricity [1], is still discussable. It is generally accepted that the occurrence of a potential difference in living systems is primarily due to a complex of physicochemical processes that ensure the maintenance of an uneven distribution of ions at the level of cells, tissues and the body.

The main electrical characteristic of the cell is the membrane potential, which arises primarily as a result of diffusion and the active process of ion transfer between the extracellular environment and intracellular compartments [2]. Ions K^+, Na^+, Ca^{2+}, Mg^{2+}, NO_3^-, $C1^-$, $H_2PO_4^-$, SO_4^- are most susceptible to active transport. Many other organic substances that are mobile within cells and tissues also carry charges, such as organic acids, amino acids, adenosine phosphates, etc. [3]. Potential differences between plant tissues and organs, generated as a result of electrogenic active transport, are determined by the physiological state and are divided into potentials of resting, action, damage, and flow [4]. Bioelectric potential (BEP) gradients result from the flow of metabolic reactions in the entire plant organism [3].

Thus, ion diffusion, concentration effects and the operation of ion pumps lead to the appearance of an electric current in plant organisms during their vital activity [5]. The electrogenic properties are most intense in the root environment—plant system, which is associated with the input and transport of ions in the process of mineral nutrition [6]. For example, the resting potential of cells of higher plants varies on average within 50-120 mV [7], while the bioelectric potential in the root zone can reach 700 mV [8].

Electroactive bacteria. Along with the diffusion of ions, which accompanies the vital activity of plants, the separation and movement of charges in the root-inhabited environment can be carried out by electroactive bacteria. In the process of development, rhizosphere microorganisms are able to oxidize organic substances secreted by the roots, synthesizing carbon dioxide, H^+ protons, and e^- electrons [9]. The transformation of the energy of chemical bonds of organic substances into electrical energy is the basis of a biotechnological device, a microbial fuel cell. In it, the generated electrons, under the action of the redox potential difference, move along the external circuit to the opposite electrode where they combine with protons that have migrated, for example, through an ion-selective membrane, and oxygen, forming water [10].

The transport of electrons from electrochemically active bacteria to the electrode surface can be carried out both directly in direct contact with the electrode, and with the help of electrically conductive processes (pilae) or mediators [11]. In particular, the transfer of electrons to the anode from bacteria of the species *Shewanella* and *Geobacter* is carried out both directly and using pili [12], while *Pseudomonas* secrete mediators (flavins) [13].

Currently, there are many species of bacteria [14] that are applicable in microbial fuel cells. Bacteria potentially capable of carrying out electrochemical reactions in the root environment were identified by the method of fluorescent in situ hybridization on plant roots, These are *Geobacter serreducens*, *Geobacter metallireducens*, *Geobacter grbiciae*, *Geobacter hydrogenophilus*, *Ruminococcus bromii*, *Clostridium sporosphaeroides* and *Clostridium leptum* [15]. It has been determined that *Shewanella putrefaciens* uses lactate, pyruvate, and formate as an electron donor [16], *Clostridium butyricum* and *Clostridium beijerinckii* use glucose, starch, lactate [17], *Rhodopseudomonas palustris* uses acetate, lactate, valerate, fumarate, ethanol, glycerol [18], *Geobacter serreducens* [19], *Geobacter sulfurreducens* [20] and *Geobacter metallireducens* [21] use acetate, *Rhodoferax ferrireducens* [22], *Alcaligenes faecalis*, *Enterococcus gallinarum* and *Pseudomonas aeruginosa* [23] use glucose, *Enterobacter cloacae* uses cellulose [24]. Most of the these compounds are present in the root environment as biota waste products and serve as an energy resource for electrochemically active bacteria.

Electric potential gradient in a root environment. Chemical reactions in the root environment, occurring as a result of the vital activity of plants and associated microorganisms, also serve as a source of electrons and ions [9, 25, 26]. Ions and electrons, formed in the process of redox reactions, diffuse through the root-inhabited medium, leading to charge separation. As a result, a gradient of electric potentials is established in the soil or soil substitute, associated with differences in the concentrations of charged substances [27, 28].

1. Difference of soil electrical potentials during plant growth of spring barley (*Hordeum vulgare* L.) cv. Leningradskii

Electrode number	Distance from the soil surface to the electrode, mm	Days							
		0-5 (seedlings)		5-13 (tillering)		13-18 (stem extension)		18-51 (earing)	
		MVD, mV	DR, mV	MVD, mV	DR, mV	MVD, mV	DR, mV	MVD, mV	DR, mV
1	30	112	18	151	18	107	14	190	23
2	80	151	27	176	21	132	16	151	30
3	130	103	18	73	4	34	5	298	63
4	180	73	14	103	15	98	13	337	81
5	230	98	22	132	13	103	10	132	23
6	280	73	13	73	14	54	11	63	9

N o t e. MVD is the maximum voltage drop vs. the bottom electrode, DR is the dispersion range of values. as based on materials [30].

The formation of different mobile charge densities due to the diffusion and adsorption of their carriers [29] is an integral part of the metabolism that accompanies the functioning and development of plants and microorganisms. In an experiment comparing the gradient of electric potentials in soil, including that under spring barley (*Hordeum vulgare* L.) cv. Leningradsky [30] it was shown that the change in the potential difference along the soil profile is associated with the stage of plant and the development of root system (Table 1).

An electric potential gradient occurred both during the development of the root system in the soil or a soil substitute, and in the soil structure itself without plants, which indicates the presence of ion transport processes, for example, due to diffusion ith the water flow. Plants in a community with rhizoplane and rhizosphere microorganisms seem to trigger additional reactions, absorbing and releasing various organic and mineral compounds, and increase the intensity of processes in the soil.

Bioelectric measurements and design features. *Plant microbial fuel cell.* Based on the ability of microorganisms to act as catalysts for redox reactions involving the extracellular transfer of electrons from microbes to the electrode [31], a bioelectrochemical system has been developed called the plant-microbial fuel cell (PMFC) [32].

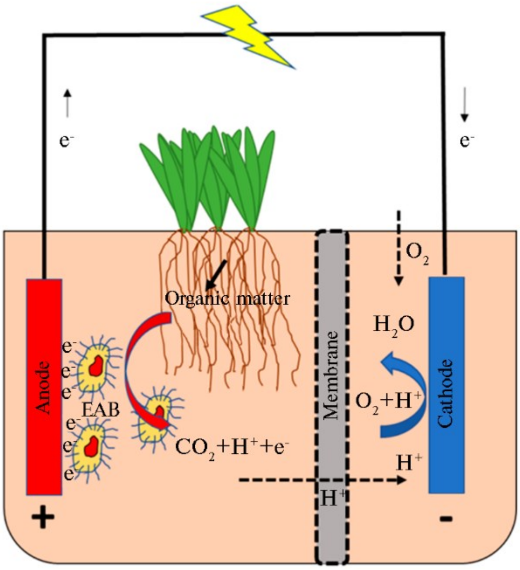

Fig. 1. Electricity generation in a plant-microbial fuel cell. Electro-active bacteria (EAB) oxidize organic matter of root exudates. As a result, carbon dioxide is formed, electrons move to the anode, and protons diffuse through the ion-exchange membrane to the cathode along the potential gradient, where water molecules are formed with the participation of electrons and oxygen molecules coming through the external circuit.

PMFC is a modification of a microbial fuel cell and, in addition to a cathode and an anode with microorganisms placed on it, includes living plants that produce rhizodeposites, the substrates for electroactive bacteria (Fig. 1). The most common configuration of the PMFC device consists of an anode chamber, an ion-selective membrane, and a cathode chamber.

In the anode chamber, microorganisms, as catalysts for the oxidation process, convert organic substances secreted by the roots according to the reaction $C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 24e^- + 24H^+$. Getting to the anode, the electrons move along the external circuit to the cathode. Protons migrate through the ion-selective membrane into the cathode chamber, where, with the participation of electrons, oxygen is reduced to form water molecules [33].

Currently, the direction of PMFC is actively developing, various modifications of the device are being created, aimed at increasing the efficiency and electrical characteristics. For example, to reduce the proton transfer distance between the electrodes, a flat plate configuration has been developed: a cation exchange membrane is placed in it between closely spaced anode and cathode chambers. The power of such PMFC was 240 mW/m^2 during long-term operation for 151 days [34]. To simplify integration into the natural environment, a model was developed in which the anode and cathode are combined into a single unit in the form of a tube. The maximum output power for this option was 72 mW/m^2 [35].

A promising way for using PMFCs is their combination with significant production processes. For example, it is possible to introduce such fuel cells into wastewater treatment systems [36] and into agricultural production.

Biocompatible electrode systems. One of the most important characteristics of the BES operation efficiency is electrode materials. In addition to high electrical performance, they must have the properties of chemical stability and biocompatibility. Most often, carbon-based materials are used as electrodes (anode and cathode) (Table 2): graphite felt, fabric, granules, rod, carbon paper, reticulated vitreous carbon [37].

2. Electrodes used in plant-microbial fuel cells (PMFC)

PMFC	Anode	Cathode	Plant species	Substrate	Output power	Reference
5 liter pots	Graphite granules	Platinum coated carbon sheet (0.4 mg/cm^2)	*Cyperus papyrus nanus* L), *Wachendorfia thyrsiflora* Burm.	Soil mix, sludge	1036±59 mW/m^3 (*Wachendorfia thyrsiflora* Burm.), 510±92 mW/m^3 (*Cyperus papyrus* L.)	[38]
Glass cylinders	Graphite granules	Graphite felt	*Glyceria maxima* Hartm.	Hoagland's solution with potassium phosphate buffer (8 mmol/l)	0.39 W/m^2	[15]
Tube-like form	Graphite felt and graphite granules	Graphite felt	*Glyceria maxima* Hartm.	Hoagland's solution rich in ammonium	10 mW/m^2 for felt, 12 mW/m^2 for granules	[35]
Flat porous plates	Three layers of graphite felt	One layer of graphite felt	*Spartina anglica* Hubbard	Nitrate-free, ammonium-rich medium for plant growth	679 mW/m^2	[39]
For growing on the roof	Graphite granules	Graphite felt	*Spartina anglica* Hubbard)	Soil mix and rainwater	88 mW/m^2	[34]

In the RMFCs based on papyrus (*Cyperus papyrus nanus* L.) and red root (*Wachendorfia thyrsiflora* Burm.), graphite granules served as the anode and a carbon sheet as the cathode. The large area of contact between the electrodes and plant roots provided in this variant and the availability of oxygen for cathodic reactions of water formation resulted in obtaining high output power values up to 1036±59 mW/m^3 [38]. In research using mannik (*Glyceria maxima* Hartm.), the influence of the electrode material on the internal resistance of the system which mainly consists of the resistances of the anode and membrane [40], was studied. Therefore, a suitable biocompatible anode electrode plays a very important role in reducing energy losses. The maximum energy production in the proposed variant of a tube-like PMFC was 10 mW/m^2 for graphite felt as an anode and 12 mW/m^2 for graphite granules [35]. The use of a biocathode, on which the reduction of oxygen is catalyzed by microorganisms, is promising. With its use, electricity generation was increased to 679 mW/m^2 in the cordgrass (*Spartina anglica* Hubbard) PMFC [39]. In real applications, such as the growing trend of growing plants on roofs, the maximum achieved power of PMFC was 88 mW/m^2 compared to 440 mW/m^2 obtained in a laboratory installation [41], which is most likely due to changes in the properties of the substrate as a result of weather conditions. Therefore, for use in natural conditions, electrode systems still need to be modified, reducing their area and resistance and increasing tolerance to external factors.

Electrical parameters. The measured characteristic, reflecting the bioelectrical activity of the root system and associated microorganisms and the course of metabolic processes in the root environment, is the electrical voltage U (V), determined by Ohm law: $U = \varepsilon - I \cdot r$. The ε parameter characterizes the electromotive force (EMF) of the BES that is due to external forces for moving charge. The product of the current strength I and the internal resistance of the system r determines the voltage drop inside the system. From this it follows that the performance of the BES and its output characteristics are highly dependent on the ability of the nutrient medium to pass an electric current. One of the most common ways to reduce the effect of internal resistance is to reduce the distance between the electrodes, that is, the gap over which the charge must be transferred

[42].

3. Generated electrical power of plant-microbial fuel cells for various plants and substrates

Plant species	Substrates	Power density, mW/m^2	Reference
Chlorophytum comosum Thunberg	Soil	18	[45]
Phragmites australis Cavanilles	Glucose + sodium acetate	43	[46]
Lactuca sativa L.	Nutrient solution	54	[47]
Ipomoea aquatic L.	Anaerobic sludge and nutrient substances	55	[48]
Brassica juncea L.	Compost potting mix	70	[49]
Glyceria maxima Hartm.	Graphite granules	80	[50]
Trigonella foenumgraecum L.	Soil	80	[49]
Puccinellia distans Jacq.	Soil mixtures	84	[51]
Typha latifolia L.	Synthetic waste water	93	[52]
Sporobolus arabicus Boiss., *Cynodon dactylon* L.	Soil	120	[53]
Oryza sativa L.	Rice fields	140	[54]
Pennisétum setaceum Forsskal	Red soil	163	[55]
Elodea Michaux	Mixed culture sludge	185	[56]
Canna stuttgart L.	Marine sediment	223	[49]
B*Eichhornia crassipes* Mart.	Precipitation	225	[57]
Chrysopogon zizanioides L.	Garden soil	242	[58]
Canna indica L.	Fermented manure	320	[59]
Lemna L.	Carbon sources and drinking water	380	[60]
Sporobolus anglicus Hubbard	Soil	679	[61]

For an effective study of electrical phenomena in a living organism and their environment, the method of diverting electrical potentials must satisfy the following conditions: 1) ensure reliable electrical contact of the electrode with the object under study, 2) exclude the possibility of the occurrence of polarization potentials, 3) take into account the electrokinetic phenomena that occur in the root-inhabited environment, 4) exclude the possibility of damage to the biological object [43]. A method for measuring the potential difference generated in the system root habitat—plants that satisfies these conditions was proposed by T.E. Kuleshova et al. [44]. It is based on a non-damaging, non-invasive method of providing surface electrical contact between the root system and the electrodes. The rate of obtaining electrical energy using BES based on plants and microorganisms is characterized by units of electrical power $P = I \cdot U$ and, as a rule, is normalized to the area occupied by plants. Table 3 presents some of the obtained power values for RMTE with various configurations, plant objects and nutrient media. Despite the fact that BES are currently low-power devices, they have a number of unique properties that make it possible to provide environmentally friendly autonomous energy in a reproducible way, which has great application prospects.

The role of environmental factors. Environmental parameters are significant for vital activity of plants and the accompanying microflora, including bioelectrogenesis. The most significant factors influencing the functioning of the BES are the composition and conditions of the root and light environments.

Influence of the composition of the root environment. Based on the electrical activity of plants and rhizospheric bacteria, BES uses a variety of root habitats, including soils of agricultural, forest, and wetlands, soil substitutes, as well as sand, clay, compost, silt, salt marshes, etc. [47, 62, 63]. In this case, the state and concentrations of the components of the nutrient medium of plants (apparently, as well as the properties of the electrolyte in a galvanic cell) play a decisive role in the output electrical characteristics of the BES.

The main "fuel" oxidized by electrochemically active bacteria is rhizodeposits: about 20-40% of all photosynthesized carbon enters the root environment in various forms in the form of root exudates, metabolites, and dead plant parts [64]. Organic compounds excreted by the roots mainly include organic acids, phenols, sugars and amino acids, and macromolecular compounds such as polysaccharides and proteins [65]. Their composition depends on the plant species, carbon sequestration method, growth intensity, plant age, and environmental conditions [66].

Along with the composition of the root-inhabited medium, the mobility of ions plays a potential-forming role in charge separation. The cations H^+, H_3O^+ with 36.2 $m^2/(V \cdot s)$, NH^{4+}, K^+ with 7.6 $m^2/(V \cdot s)$, Fe^{3+} with 7 $m^2/(V \cdot s)$ and anions OH^- with 20.5 $m^2/(V \cdot s)$, Cl^- with 7.9 $m^2/(V \cdot s)$, NO^{3-} with 7.4 $m^2/(V \cdot s)$ are the most mobile [67]. In addition, the magnitude of the potential difference depends on soil moisture, including both the change in the resistance of the environment and the processes of absorption and transport of water associated with the vital activity of plants. For example, T.E. Kuleshova et al. [68] show the dependence of the electrical voltage created in the root zone on the water regime, including water-deficient conditions.

The influence of lighting conditions. It is known that light plays an important role in the formation of bioelectric potentials. For example, when the light is turned on, there is a sharp drop in the BEP of the leaf surface, and then a quick jump [69]. If one part of the leaf is illuminated and the other part is shaded, then the potential difference will vary from 50 to 100 mV [70]. This variation in metabolic potentials is associated primarily with differences in the intensity of biochemical processes in different parts of the plant.

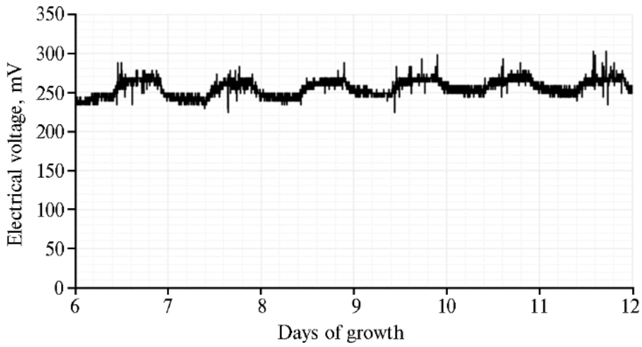

Fig. 2. Electrical voltage changes in a plant-microbial fuel cell based on *Chlorophytum comosum* Thunberg under 12-hour light and dark periods.

A change in the potential difference in response to light exposure is also noted during electroactive reactions in the root environment. It is shown that when the dark stage changes to the light one, the voltage in the root zone gradually increases by 10-15% and then evenly decreases (Fig. 2), this semi-diurnal dynamics can be described by a polynomial of the second degree. Parabolic voltage changes during the light stage of photosynthesis and stationary generation during its dark regime are most likely related to the intensity of transport of water, mineral and organic substances, depending on the formed light conditions. It is also known that the electrical resistance of the leaf surface depends both on the temperature and moisture content of the tissue, and on the mobility and concentration of ions in the tissue medium: the leaf resistance increases with wilting and decreases with watering to its original value [71].

Therefore, environmental factors play a significant role in the formation and course of bioelectric processes. A correlation was shown [72] between the dynamics of light transmission by a leaf plate and the potential difference in the root environment-plant system, which indicates the possible conversion of light energy by plant leaves into electric current in the rhizosphere.

BES application. *BES combined with plant production.* PMFC is a renewable energy source that can simultaneously produce bioelectricity and biomass in an environmentally friendly, sustainable and efficient manner [28]. The use of hybrid technology, which makes it possible to produce plant products and generate electricity by activating oxidative processes in the rhizosphere, is an innovative direction with the prospect of application in the field of autonomous automated agricultural production. The possibility of obtaining low-power energy using BES when growing plants has already been shown on some cultivated and industrial plants, including rice [73], lettuce [47], manna [15], reeds [74], goz [75], used as food, fuel, building materials, and animal feed.

BES universal device proposed by T.E. Kuleshova et al. [76] and suitable for growing vegetable crops (greens, tomatoes, cucumbers), based on thin-layer panoponic technology [77]. In such PMFC, electrode systems are placed in the cultivation tank perpendicular to the growth of the root system, thereby ensuring that the surface contact of the roots with the electrically conductive material does not damage the plants [44]. It is assumed that the formation of a gradient of electrical potentials in the BES is a consequence of the movement of ions along the root system and concentration effects, and the occurrence of EMF between the electrodes is ensured by the vital activity of plants and the electrical activity of the microbial community surrounding the root system.

The PMFC technology makes it possible to produce "green" energy almost everywhere where plants grow, and is applicable both in the natural environment and for growing crops in open ground and greenhouses, in phytotechnical complexes and regulated agroecosystems, which is especially important for areas geographically isolated from the unified power system. The agro-technological energy complex based on BES is able to provide not only environmentally friendly energy, but also high-quality plant products.

Biobatteries in the environment. Biobatteries are environmentally friendly integrated bioelectrochemical systems that convert chemical energy into electricity from or with the help of bioresources. Unlike conventional batteries which cause pollution [78], biobatteries are considered a sustainable and renewable source of energy. However, life cycle assessment (LCA) of these systems before and after their implementation is still a challenge [79] and depends on the types of material used in the fabrication of the structure.

Biobatteries include anode and cathode electrode systems and can use various biochemical energy sources [80-84].

Biobatteries have already been tested in various plant species and under varying environmental conditions [85]. In particular, plants of the cactus family (*Opuntia* Miller) which carry out CAM photosynthesis and are applicable in arid conditions, were used [86].

In the biobattery based on a vertically integrated ceramic tube containing graphite felt as the anode material and zinc foil as the cathode, the maximum power density achieved with *Opuntia albicarpa* Scheinvar was 103.6 mW/m^2 under long-term operation conditions. The addition of ammonium nitrate ($150\ mg \cdot l^{-1} \cdot week^{-1}$) to the *Opuntia joconostle* (Weber ex Diguet) biobattery resulted in an increase in energy yield from 40 to 500 mW/m^3 [87]. The developed biobatteries were effectively used to power LEDs and digital clocks, ensuring their autonomous operation for a week [85]. The use of plants with CAM photosynthesis in BES is promising for areas with limited resources and in semi-arid territories. However, this requires large-scale studies.

Phytomonitoring and power supply of sensors. BES can perform a dual function, acting as a biosensor for phytomonitoring and providing power to environmental sensors. In the work of D. Brunelli et al. [88] PMFC has been used to track the physiological state of plants and monitor light intensity and soil moisture. The energy from the PMFC was accumulated on a supercapacitor and then used to send a signal from the sensors with an interval of 15 min.

The developed wireless sensor networks (WSN) and the Internet of things

(IoT) are of priority importance for the tasks of smart farming, continuous monitoring of the state and needs of plants [89], especially for use in areas remote from power grids. PMFCs can provide an environmentally friendly option for powering these systems. At present, the problem of low-power and intermittent energy production using BES is solved by integrating supercapacitors [90]. E. Osorio-De-La-Rosa et al. [91] demonstrated the launch of an IoT-based sensor. The cell was capable of generating 3.5 mW/cm^2 with an output voltage 0.5 V which is sufficient for batteryless operation of the sensor assembly for temperature data collection and cloud storage.

Thus, BES can be used to create low-power, unattended renewable energy sources that can partially support the vital activity of plants by supplying power to light sources, pumps, sensors for plant and environmental parameters. It can also be used in scientific research and in crop production as a biosensor for setting up growing technologies and phytomonitoring.

Wastewater treatment based on BES. The introduction of BES is also rapidly developing in the field of wastewater treatment. Compared to traditional technologies, BES are more cost-effective and sustainable, as they have the advantage of renewable bioenergy resources [92]. Various possibilities of their application are being studied, in particular, the use of electrochemically active microorganisms for the removal of organic substances and heavy metals is considered promising. BES can work productively for the oxidation of organic substances in the anode chamber, especially in relation to municipal and industrial wastewater with high chemical oxygen demand, such as brewing, food and textile [93-96]. Organic waste can also act as an electron donor for microorganisms in the reduction of heavy metals. The work of Y.V. Nancharaiah et al. [97] gives information on the reduction of Ag(I), Au(III), Co(III), Cr(VI), Cu(II), Hg(II), Se(IV) or V(V) ions in the BES cathode chamber. The recovery efficiency of Cr(VI) in the work of H. Yu et al. [98] reached 99.93%.

As a result of microbial processes in BES, nitrogen removal is also possible [99]. For example, N. Yang et al. [100] showed high removal efficiency of NH^{4+}-N (99%) and total nitrogen (TN, 99%) in BES with upflow in which microbial metabolism was enhanced to carry out simultaneous nitrification, denitrification and other bioelectrochemical reactions.

So, bioelectrochemical systems based on electroactive processes in the root environment of plants and associated microorganisms are a new promising environmentally friendly technology for generating renewable energy. The performance of bioelectrochemical systems (BES) depends on a number of factors, including genetically determined physiological characteristics of plants and their state during development, the composition and activity of the microbial community, the parameters of the root environment, environmental factors, the design of the bioreactor, the type and location of electrode systems. Despite the low output power, plant-microbial energy devices have their own niche application both in the present and in the future, providing power to environmental sensors, light sources, wireless sensor networks, the Internet of things, phytomonitoring systems in natural conditions and protected ground, remote areas, partial power supply of plant life support devices in artificial agroecosystems. Generation of "green" energy can be accompanied by the production of vegetable raw materials and wastewater treatment. Further development prospects lie in the creation of multifunctional electrical circuits that take into account the properties of the root environment and plants in order to increase the efficiency of the system and the amount of generated electricity.

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CARBON AND SILICA NANOSTRUCTURES IN THE PROTECTION OF SPRING BARLEY FROM DISEASES IN THE NORTH-WEST RUSSIA

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Abstract

Spring barley (*Hordeum vulgare* L.) is the main grain fodder crop, annually occupying about 40 % of the sown area in the North-West Russia. In recent years, there has been a clear interest in the world and domestic science to use of nanomaterials and nanotechnologies in plant protection, which is due to their unique properties and high efficiency at low concentrations. In this work, for the first time, the effect of carbon and silica sol nanocompositions on seed infection, damage to spring barley plants by root rot and leaf diseases is shown. It was determined that a stronger protective effect was manifested when using nanocompositions on the spring barley variety Ataman with a longer growing season and more susceptible to major diseases. For the first time, an additive effect has been established that enhances the protective functions of chemical or biological fungicides with the possibility of reducing their dosage when combined with nanocomposites in the treatment of seeds and vegetative plants. Our goal was to study the effectiveness of new compositions based on carbon and silica sol nanomaterials in protecting spring barley from diseases in the North-West Russia. The studies were carried out at the experimental base of the Menkovsky branch of the Agrophysical research institute (Gatchinsky District, Leningrad Province) in 2017-2018. At the first stage of research in 2017, the effectiveness of two promising nanocompositions for the protection of spring barley from root rot and leaf diseases was studied. Two experiments were carried out on Leningradsky and Ataman varieties of spring barley with different vegetation periods: on the treatment of seed material and vegetative plants with nanocompositions. The silica sol composition of NKteos was synthesized according to the original sol-gel technology based on acid hydrolysis followed by polycondensation of tetraethyl ester of orthosilicic acid or tetraethoxysilane, with the addition of macro- and microelements salts solutions and dopants — a charge of detonation nanodiamond doped with boron, or a titanium dioxide in the form of anatase to the sol. Preparation of a nanocomposition based on fullerene derivatives with methionine or threonine was carried out by dissolving microelement compounds in water and adding 0.001 % (for seed treatment) or 0.00001 % (for foliar treatment) solution of the amino acid derivative of C_{60} fullerene with threonine or with methionine. Experiment variants also included the combined use of nanocompositions with chemical and biological fungicides, as well as fungicides with silicon-containing chelated microfertilizer. Grain contamination with phytopathogens was determined using nutrient media. The development of root rot was t assessed in the phases of germination, tillering, budding and heading, leaf diseases — in the beginning of barley earing, then in 10, 20 and 30 days. At the second stage of research in 2018, the effectiveness of the technological scheme for the use of new nanocompositions in the protection of spring barley of the Leningradsky variety from diseases was evaluated. The experiment included two blocks: the treatment with nanocompositions of seeds, the treatment of seeds and vegetative plants. It is shown that the studied nanocompositions in their pure form turned out to be ineffective in protecting spring barley from root rot and leaf diseases. The decrease in the development of root rot on the early ripe variety Leningradsky did not exceed 5.3 %, on the variety Ataman it was 15.3-57.7 % ($p < 0.05$). The development of the main disease of the crop — helminthosporium spots on the two upper leaves of barley plants of the Leningradsky variety decreased by 16-22 %, of the Ataman variety — by 20-42 % ($p < 0.05$). The results of seed treatment allow us to assume that the effect of the silica sols composition is longer, since it extended to the development of helminthosporium leaf spots (decrease in damage by 7.5-15.4 %, $p < 0.05$ compared to control) and is due to the ability to activate plant metabolism and immunity. The effect of the nanocomposition based on the fullerene C_{60}-methionine derivative is more apparent due to a decrease in seed infection and primary signs of infection during the emergence of barley seedlings. The most effective for the protection of spring barley from root and leaf diseases was the combined treatment of seeds with a silica sol nanocomposition and the chemical fungicide Insure™ Perform, KS, followed by a triple treatment of vegetative plants with a nanocomposition based on a C_{60}-threonine derivative and a single treatment with the chemical fungicide Zantara, CE. Reducing the dose of a chemical preparation is advisable only if a weak manifestation of the disease is expected. High biological and economic efficiency, comparable to the result of fungicidal treatment with 100 % application rate of the preparation, was ensured by the combined use of silicon containing chelate microfertilizer SCM-G and fungicide (50 % application rate), as well as nanocomposition based of the C_{60} fullerene amino acid derivatives with methionine and fungicide (50 % application rate).

Keywords: *Hordeum vulgare* L., spring barley, root rot, leaf diseases, plant protection products, fungicides, nanomaterials, fullerene C_{60}, amino acid derivatives, C_{60} with methionine, C_{60} with threonine, silica sol, tetraethoxysilane. dopants, charge of detonation nanodiamond, titanium dioxide, anatase

In recent years, there has been a clear interest in the use of nanomaterials and nanotechnologies in agriculture, due to their unique properties and high efficiency at low concentrations [1-3]. Due to their small size and electrical neutrality, nanomaterials easily penetrate the cell membrane, and due to their large specific surface, they have a high reactivity [4-6]. Promising nanomaterials that can potentially increase plant resistance to biotic and abiotic stressors include carbon and silica nanocomposites [7-8].

Carbon nanocomposites are compositions based on water-soluble derivatives of C_{60} or C_{70} fullerene and a number of microelements, as well as physiologically active compounds [9, 10]. Recently, the number of new synthesized amino-, carboxy-, polyhydroxy- and other fullerene derivatives, which are convex closed polyhedra, composed of an even number of three-coordinated carbon atoms, has been growing [11]. Since their discovery in 1985, which was awarded the Nobel Prize in Chemistry in 1994, fullerenes and their derivatives have been increasingly used in engineering, in medicinal chemistry and in cosmetology [12-14].

Silica-based mesoporous supports, the so-called mesoporous silicate materials, have been the subject of much research since Mobil Oil (USA) synthesized mesoporous silica materials in 1992 with an ordered mesopore structure, narrow pore size distribution, high specific surface [15, 16]. In contrast to the microporous structure of zeolites already known at that time (pore diameter of about 1.5 nm), mesoporous materials have a pore size of about 3-5 nm. This class of materials served as a prototype for the further development of silica-based nanoporous structures, including silica matrices created using the original sol-gel technology [17] with the inclusion of organic molecules, nanosized particles of metals, their oxides, nanodiamonds, carbon nanotubes, which provide enhanced functional characteristics silica sols.

At the Agrophysical Research Institute (API), together with the St. Petersburg State University (SPbSU), the First St. Petersburg State Medical University (PSPbSMU), the Institute of Silicate Chemistry (ISC RAS), biologically active nanocompositions have been developed, e.g., complex microfertilizers based on water-soluble derivatives of C_{60} fullerenes [18, 19] and silica-sol nanocomposites based on tetraethoxysilane with solutions of macro- and microelements doped with a mixture of detonation nanodiamond or titanium dioxide in the form of anatase [20-22]. Aqueous suspensions of detonation nanodiamond or charge are of particular interest as precursors of composite materials or as biologically active additives, since colloidal carbon nanoparticles in an aqueous dispersion medium are the most chemically active [23]. Along with this, detonation nanodiamond and its charge doped with boron, as well as a number of metal nanoparticles (magnetized iron, aluminum, copper, gold, silver, silicon, zinc and zinc oxide, titanium dioxide, cerium oxide, etc.) have biocidal properties against bacteria, viruses and micromycetes. Their inclusion in the composition of silica sols is promising for enhancing the phytoprotective function [24-26].

The creation of preparations was based on the following principles: environmental safety, simplicity of the technological process, low cost, efficiency in small doses and availability to plants. Under controlled and field conditions, the effect of foliar treatment with nanocomposites based on fullerene derivatives on the growth, development, grain productivity of ereals and vegetable crops, their resistance to oxidative stress has been established [18, 19], and the ability to form a film (shell) around the seed under seed treatment with tetraethoxysilane-based silica sols has been shown [21, 22]. The presence of a shell with a selected qualitative and quantitative composition of components on the surface of the seeds ensured the stimulation of plant growth at the initial stages of development [21, 22].

With the presence of the properties of immunomodulators and adaptogens, there are great prospects for the use of the obtained nanocompositions in the protection of cultivated plants from phytopathogens. The first data on the positive effect of the treatment of spring barley seeds with C_{60} fullerene derivatives with methionine and threonine, as well as their nanocompositions on plant resistance to damage by the root rot pathogen Cochliobolus sativus (S. Ito & Kurib.) Drechsler ex Dastur [20], were obtained. The mechanism of their action is not associated with the regulation of the number of microorganisms on the surface of seeds. Apparently, it is due to the ability to activate the metabolism and antioxidant abilities of plants [20]. Additionally, it was found that the sol-gel composition are capable of increasing plant resistance to phytopathogens not only by activating plant immunity, but also by regulating the abundance of microorganisms, including potential pathogenes, on the surface of seeds [20].

Currently, the prospects for the use of nanopreparations in the protection of plants from harmful organisms are being extensively studied [27, 28]. In the Northwestern region of Russia, such studies are in demand for many agricultural crops, including spring barley, which annually occupies approx. 40% of the sown area [29].

In this work, for the first time, the effect of carbon and silica-sol nanocompositions on seed infection, as well as damage to spring barley plants by root rot and leaf diseases, is shown. It is shown that a stronger protective effect was manifested when using nano-compositions on the spring barley variety Ataman with a longer vegetation period and greater susceptibility to major diseases. For the first time, the presence of an additive effect was established in the combined treatment of seeds and vegetative plants with nano-compounds with chemical or biological fungicides potentially reducing the dosage of the preparations.

Our goal was to study the effectiveness of new compositions based on carbon and silica nanomaterials in the protection of spring barley from diseases in the North-West of the Russian Federation.

Materials and methods. The studies were carried out at the experimental base of the Menkovsky branch of the Agrophysical Institute (API, Gatchinsky district, Leningrad Province) in 2017-2018. The soil of the experimental fields is soddy-slightly podzolic sandy loam. The 23 cm soil layer is arable, the content of organic matter is 0.70-0.77%, mobile compounds of phosphorus and potassium (according to Kirsanov) are 266-298 and 153-167 mg/kg, respectively, pH_{KCl} 5.1-5.7.

At the first stage of work in 2017, the effectiveness of two promising nanocompositions for the protection of spring barley (*Hordeum vulgare* L.) from root rot and leaf diseases was assessed. In accordance with the methodological guidelines [30], two experiments were performed on cv. Leningradsky (64-75 days) and cv. Ataman (79-98 days) with different vegetation periods, the seeds and vegetative plants were treated with nanocomposites. To evaluate the efficiency of pre-sowing application of nanocomposites, the following treatments were used: 1 — water (control); 2 — Insure Perform, KS (Insure™ Perform, BASF, Germany; 0.4 l/t); 3 — Inshur Perform, KS (0.2 l/t); 4 — silica-ash composition based on 1 wt.% tetraethoxysilane with macro- and microelements and nanodiamond charge from detonation (NKteoa) (1.0 l/t); 5 — composition of fullerene derivative with methionine and trace elements (NFm) (1.0 l/t); 6 — NKteoa (1.0 l/t) + Inshur Perform, KS (0.4 l/t); 7 — NKteoa (1.0 l/t) + Inshur Perform, KS (0.2 l/t); 8 — NFm (1.0 l/t) + Inshur Perform, KS (0.4 l/t); 9 — NFm (1.0 l/t) + Inshur Perform, KS (0.2 l/t); 10 — Vitaplan, joint venture (AgroBioTechnology, Russia; 20 g/t); 11 — NKteoa (1.0 l/t) + Vitaplan, SP (20 g/t); 12 — NFm (1.0 l/t) + Vitaplan, SP (20 g/t).

The NKteoa silica-ash composition was synthesized according to the original sol-gel technology (21, 22) based on acid hydrolysis followed by polycondensation of tetraethyl ester of orthosilicic acid (TEOA), with the addition of solutions of salts of macro- and microelements and dopants (a mixture of detonation nanodiamond alloyed with boron, or titanium dioxide in the form of anatase). The composition of silica sols is 1 vol.% TEOA + microelement solution (pH 2-3) + 0.1% charge in the form of anatase with boron or 0.1% TiO_2. The solution of macro- and microelements included N, P, K, Ca, Mg, S, Fe, B, Zn, Cu, and Mn compounds [21, 22]. The mixture of detonation nanodiamond was enriched with boron directly during the in situ explosion and contained 0.96% boron, 14.7% detonation nanodiamond, 80.84% non-diamond forms of carbon, and 0.96% non-combustible impurities [25].

The preparation of a nanocomposition based on fullerene derivatives with threonine (NFtr) or methionine (NFm) was carried out by dissolving microelement compounds in water and adding 0.001% (for seed treatment) or 0.00001% (for foliar treatment) of a solution of the amino acid derivative of C_{60} fullerene with threonine or methionine [9].

Sol concentrations, as well as solutions of C_{60} fullerene amino acid derivatives with threonine or methionine (10.0 mg/l when treating seeds and 0.1 mg/l when treating vegetative plants), were selected based on the results of assessing the reactions of plants in vegetation experiments at the agro-biopolygon of the Agrophysical Institute (API) [19-21].

Inshur Perform, KS was used as a chemical fungicide for seed treatment (reference), the flow rate of the working fluid was 10 l/t. In continuation of our previous studies [31, 32], the experiment included the treatment of seed with biofungicide Vitaplan, SP.

Pre-sowing treatment of barley seeds was carried out by a previously developed method [21]. The seeds were soaked with constant stirring using a UED-20 magnetic stirrer (UED Group, Russia) in water (control) or in a silica sol solution for 10 min, after which they were dried at room temperature in air until dry and then at 30 °C for 50 min in an oven ShS-80-01 MK SPU (OAO Smolenskoye SKTB SPU, Russia). The treated seeds were stored at room temperature for 2 days before sowing. The treatment of seed material with chemical and biological preparations was carried out using a Hege 11 unit (Wintersteiger AG, Austria), designed for wet dressing of small batches of seeds.

The layout of the test plots (2 m^2) was randomized, with a 4-fold repetition. The predecessor was winter rye (*Secale cereale* L.) cv. Slavia. The seeds were sown manually.

In this and all experiments described below, tillage included autumn plowing and pre-sowing cultivation. Before sowing, ammonium nitrate was added at the rate of 60 kg/ha of active weight; in the tillering phase of barley, the herbicide Sekator, VDG (Sekator, Bayer CropScience, Germany; 0.15 kg/ha) was treated. The seeding rate is 5 million germinating seeds/ha. For spring barley yield assessment, sheaves were collected from a 1 m^2 of each plot at the phase of full ripeness. Under laboratory conditions, productive stem density, grain weight per ear and 1000-grain weight were measured.

Grain contamination with phytopathogens was determined using nutrient media [33]. Accounting for the development of root rots was carried out according to the relevant guidelines (30) on 30 plants from each plot in the phases of germination, tillering, budding and heading.

To evaluate the effectiveness of plant treatment with nanocomposites, experimental design was as follows: 1 — control (treatment of plants with water); 2 — Zantara, CE (Zantara, Bayer CropScience, Germany; 0.8 l/ha); 3 — Zantara, CE (0.4 l/ha); 4 — silicon-containing chelate microfertilizer KKhM-G (3.0 l/ha); 5 — NFm (1.0 l/ha); 6 — NFtr (1.0 l/ha); 7 — KKhM-G (3.0 l/ha) + Zantara, CE (0.8 l/ha); 8 — KKhM-G (3.0 l/ha) + Zantara, CE (0.4 l/ha); 9 — NFm (1.0 l/ha) + Zantara, CE (0.8 l/ha); 10 — NFm (1.0 l/ha) + Zantara, CE (0.4 l/ha); 11 — NFtr (1.0 l/ha) + Zantara, CE (0.8 l/ha); 12 — NFtr (1.0 l/ha) + Zantara, CE (0.4 l/ha).

Silicon-containing chelated microfertilizer KKhM-G, developed at the API (34), is an environmentally friendly biostimulant and plant resistance inductor, the main active components of which are silicon, microelements and a chelating agent (humic acids isolated from high-moor peat of a low degree of decomposition). The mass fraction for humic acids was 0.12, for Fe 9.7, B 2.07, Mn 1.66, Si 5.33, Cu 1.60, Co 1.76, Zn 1.66, Mo 3.85, S 8.17%. The working solution was applied at a rate of 300 l/ha.

As a reference, a chemical fungicide for the treatment of vegetative plants Zantara, CE, was used, the flow rate of the working solution was 300 l/ha. The repetition of treatments for microfertilizers and nano-compositions is 3-fold, starting from the tillering phase with an interval of 7 days, for a chemical fungicide - once in the phase of the beginning of barley heading.

A randomized layout of 10 m^2 plots with a 4-fold repetition was used, the predecessor was winter rye cv. Slavia. Sowing was carried out with a Lemken soliter seeder (Lemken GmbH & Co. KG, Germany), the seeding rate was 5 million germinating seeds/ha. The plants were treated using a Solo 475P knapsack sprayer (Solo Kleinmotoren GmbH, Germany).

Leaf diseases were recorded at the beginning of earing, then after 10, 20 and 30 days on 10 stems in three sites on each plot in accordance with the guidelines [30].

At the second stage of research in 2018, the effectiveness of the technological scheme for the use of new nanocompositions in protecting spring barley of the Leningradsky variety from diseases was evaluated. The experiment included two blocks, the first with the treatment of seed material with nanocomposites, the second with the treatment of seeds and vegetative plants.

Block A included treatment of seeds with preparations and vegetative plants with water: 1 — control (seed treatment with water) + water; 2 — Inshur Perform, KS (0.4 l/t) + water; 3 — Inshur Perform, KS (0.2 l/t) + water; 4 —

NKteos (1.0 l/t) + water; 5 — NKteos (1.0 l/t) + Inshur Perform, KS (0.4 l/t) + water; 6 — NKteos (1.0 l/t) + Inshur Perform, KS (0.2 l/t) + water. Block B included treatments of seeds with preparations and vegetative plants with a solution of a nanocomposition based on the amino acid derivative of C_{60} fullerene with threonine (NFtr) at a previously selected concentration of 0.1 mg/l: 7 — treatment of seeds with water + treatment of plants with NFtr (1.0 l /ha); 8 — Inshur Perform, SC (0.4 l/t) + NFtr (1.0 l/ha); 9 — Inshur Perform, SC (0.2 l/t) + NFtr (1.0 l/ha); 10 — NKteoa (1.0 l/t) + NFtr (1.0 l/ha); 11 — NKteoa (1.0 l/t) + Inshur Perform, KS (0.4 l/t) + NFtr (1.0 l/ha); 12 — NKteoa (1.0 l/t) + Inshur Perform, KS (0.4 l/t) + NFtr (1.0 l/ha).

The preparation of nanocompositions, seed and plant treatments were carried out as in previous experiments. Plants were treated 3-fold, starting from the tillering phase, with a 7-day interval. The plots (2 m^2 each, with a 4-fold repetition) were randomized. The predecessor was winter wheat cv. Moskovskaya 56. Seeding manual. The plants were treated using a Solo 475P knapsack sprayer.

Statistical processing of the obtained data was carried out using analysis of variance in the Statistica 6.0 program (StatSoft, Inc., USA). In the calculations, parametric statistics methods were used based on mean values (M) and standard errors of the means (±SEM), 95% confidence intervals, and the least significant difference of LSD at $p < 0.05$ (LSD_{05}).

Results. Seed infection acts as the primary focus of infection of the root system of plants. Phytoexamination of seed material showed an additive effect from the combined use of the chemical fungicide Inshur Perform, KS in full application rate with the tested carbon and silica nanocompositions against the fungus *Cochliobolus sativus* (S. Ito & Kurib.) Drechsler ex Dastur, the main causative agent of helminthosporium-fusarium root rot (Table 1). In addition, the high efficiency of the silica-sol composition against fungi of the genus Fusarium (85.7-100%) was noted. The effects of the use of the biological product Vitaplan, SP, including in combination with nanocompositions, were unstable and concerned mainly Fusarium spp. and Alternaria spp. The results obtained are consistent with previously published data on the practical absence of a biocidal effect of the studied carbon and silica compositions against *C. sativus* [20].

1. Efficiency of nanocompounds, chemical and biological fungicides and their combinations against seed infection in spring barley (*Hordeum vulgare* L.) ($n = 10$, M±SEM; Leningrad Province, 2017)

Variant	Cultivar	Microbiota					
		pathogenic				saprotrophic	
		Cochliobolus sativus		*Fusarium* spp.		*Alternaria* spp.	
		DI, %	BE, %	DI, %	BE, %	DI, %	BE, %
1. Control (water)	L	23.0±3.26		3.3±0.87		37.0±5.24	
	A	47.0±4.87		7.0±2.04		41.0±6.02	
2. Inshur Perform, KS (0.4 l/g)	L	8.0±2.22	65.2	0.0	100.0	4.0±0.79	89.2
	A	29.0±4.41	38.3	0.0	100.0	19.0±5.25	53.7
3. Inshur Perform, KS (0.2 l/g)	L	22.0±2.98	4.3	0.0	100.0	13.0±2.88	64.9
	A	43.0±4.68	8.5	9.0±2.54	0.0	38.0±4.54	7.3
4. NKteoa (1.0 l/g)	L	40.0±4.75	0.0	0.0	100.0	34.0±4.87	8.1
	A	45.0±5.25	4.3	1.0±0.25	85.7	48.0±5.81	0.0
5. NFm (1.0 l/g)	L	48.0±5.98	0.0	6.0±1.94	0.0	17.0±2.91	54.1
	A	43.0±4.75	8.5	5.0±1.65	28.6	47.0±5.64	0.0
6. NKteoa (1.0 l/g) + Inshur Perform, KS (0.4 l/g)	L	2.0±0.31	91.3	0.0	100.0	27.0±5.04	27.0
	A	29.0±4.47	38.3	0.0	100.0	8.0±2.34	80.5
7. NKteoa (1.0 l/g) + Inshur Perform, KS (0.2 l/g)	L	17.0±2.88	26.1	0.0	100.0	16.0±2.88	56.8
	A	52.0±5.03	0.0	2.0±0.38	71.4	18.0±3.05	56.1
8. NFm (1.0 l/g) + Inshur Perform, KS (0.4 l/g)	L	5.0±1.14	78.3	0.0	100.0	15.0±2.54	59.5
	A	22.0±3.05	53.2	4.0±0.95	42.9	6.0±1.85	85.4
9. NFm (1.0 l/g) + Inshur Perform, KS (0.2 l/g)	L	21.0±3.47	8.7	3.0±0.74	9.1	7.0±2.03	81,1
	A	57.0±5.54	0.0	10.0±3.01	0.0	4.0±0.86	90,2
10. Vitaplan, SP (20 g/t)	L	12.0±2.61	47.8	0.0	100.0	14.0±2.54	62,2
	A	54.0±5.35	0.0	9.0±2.74	0.0	25.0±4.05	39,0

Continued Table 1

11. NKteoa (1.0 l/t) + Vitaplan, SP (20 g/t)	L	41.0±4.44	0.0	0.0	100.0	19.0±3.02	48,6
	A	59.0±5.89	0.0	3.0±0.65	57.1	35.0±4.88	14,6
12. NFm (1.0 l/t) + Vitaplan, SP (20 g/t)	L	24.0±3.35	0.0	0.0	100.0	32.0±4.45	13,5
	A	85.0±8.61	0.0	4.0±0.84	42.9	4.0±0.78	90,2
LSD_{05} (cultivar)		4,44		2.10		5.25	
LSD_{05} (preparations)		5,35		3.03		7.02	
LSD_{05} (cultivar, preparations)		5,91		4.21		8.10	

N o t e. L — Leningradsky, A — Ataman; DI — disease incidence, BE — biological effectiveness. Each sample consists of 10 grains. For detailed descriptions, see the Materials and methods section.

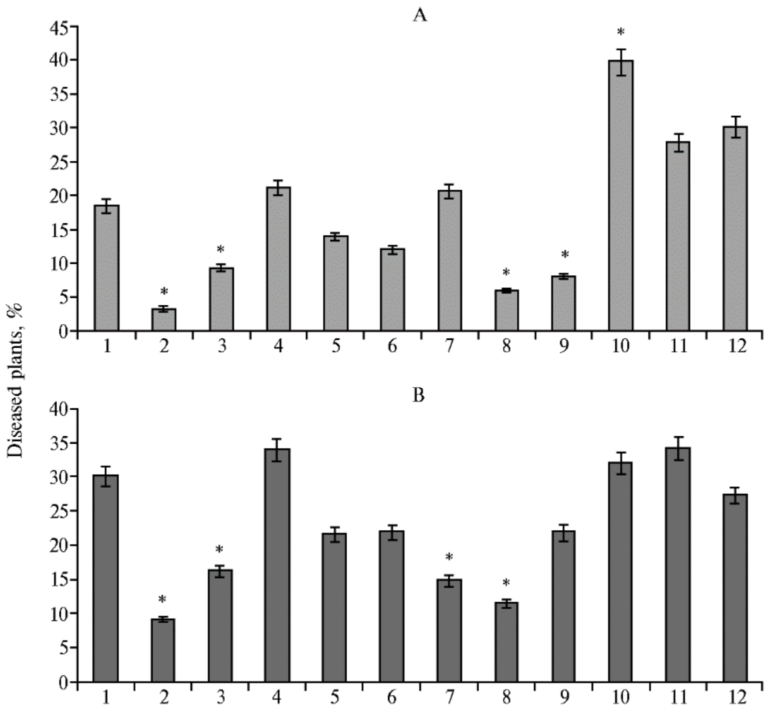

Fig. 1. Manifestation of primary root rot symptoms on plants of spring barley (*Hordeum vulgare* L.) varieties Leningradsky (A) **and Ataman** (B) **after seed treatment with nanocompounds, chemical, biological fungicides and their combinations:** 1 — control (water); 2 — Inshur Perform, KS (0.4 l/t); 3 — Inshur Perform, KS (0.2 l/t); 4 — silica composition NKteoa (1.0 l/t); 5 — composition of a fullerene derivative with methionine and NFm microelements (1.0 l/t); 6 — NKteoa (1.0 l/t) + Inshur Perform, KS (0.4 l/t); 7 — NKteoa (1.0 l/t) + Inshur Perform, KS (0.2 l/t); 8 — NFm (1.0 l/t) + Inshur Perform, KS (0.4 l/t); 9 — NFm (1.0 l/t) + Inshur Perform, KS (0.2 l/t); 10 — Vitaplan, SP (20 g/t); 11 — NKteoa (1.0 l/t) + Vitaplan, SP (20 g/t); 12 — NFm (1.0 l/t) + Vitaplan, SP (20 g/t) (n = 4, M±SEM; Leningrad Province, 2017). Each sample consists of 10 grains. For detailed descriptions, see the Materials and methods section.
* Differences from control are statistically significant at $p < 0.05$.

The primary symptoms of damage to seedlings of spring barley with root rot were the least manifested when seeds were treated with a chemical protectant at the full rate of application. The absence of a protective effect already at this stage was seen in the variants with the treatment of seeds with the silica-sol nanocomposition NKteos, as well as the biological preparation separately and together with the tested nanocompositions (Fig. 1). Both varieties of barley showed a decrease in the primary infection of root rot when seeds were treated with a nanocomposition based on C_{60} fullerene with methionine (24.9 and 28.2%), and even more so in combination with a chemical disinfectant (55.7 and 39.0%).

A subsequent assessment of the development of root rot showed that the most powerful and long-lasting protective effect was provided by the treatment of seeds with the chemical agent Inshur Perform, KS at 0.4 l/t. Its efficiency was

100, 76.3, 35.3% and 100, 84.6, 47.2%, respectively, for varieties Leningradsky and Ataman at tillering, stem extention and heading. A twofold decrease in the dosage led to a significant loss of the effectiveness of the chemical.

2. Efficiency of seed treatment with nanocompounds, chemical, biological fungicides and their combinations against root rot in spring barley (*Hordeum vulgare* L.) varieties at different stages of plant development (*n* = 4, *M*±SEM; Leningrad Province, 2017)

Variant	Cultivar	Stage					
		tillering		stem extention		heading	
		R, %	BE, %	R, %	BE, %	R, %	BE, %
1. Control (water)	L	1.4±0.63		3.8±0.64		6.8±2.05	
	A	1.1±0.66		2.6±0.96		7.2±1.58	
2. Inshur Perform, KS (0.4 l/g)	L	0.0	100.0	0.9±0.43	76.3	4.4±1.55	35,3
	A	0.0	100.0	0.4±0.27	84.6	3.8±2.05	47,2
3. Inshur Perform, KS (0.2 l/g)	L	1.0±0.70	28.6	1.3±0.52	65.8	7.3±1.94	0,0
	A	0.0	100.0	0.3±0.36	88.5	4.5±2.50	37,5
4. NKteoa (1.0 l/g)	L	1.9±0.20	0.0	3.6±1.24	5.3	9.5±3.55	0,0
	A	1.1±0.52	0.0	1.1±0.40	57.7	6.1±1.45	15,3
5. NFm (1.0 l/g)	L	2.0±0.67	0.0	3.6±0.78	5.3	8.6±1.53	0,0
	A	0.7±0.27	36.4	1.7±0.75	34.6	6.1±1.26	15,3
6. NKteoa (1.0 l/g) + Inshur Perform,	L	0.8±0.44	42.9	1.4±0.84	63.2	7.0±1.96	0,0
KS (0.4 l/g)	A	0.0	100.0	1.2±0.33	53.8	6.8±1.47	5,5
7. NKteoa (1.0 l/g) + Inshur Perform,	L	1.9±0.13	0.0	4.3±0.96	0.0	7.0±2.17	0,0
KS (0.2 l/g)	A	0.0	100.0	0.7±0.42	73.1	6.1±1.23	15,3
8. NFm (1.0 l/g) + Inshur Perform, KS	L	0.4±0.26	71.4	1.1±0.48	71.1	3.8±1.37	44,1
(0.4 l/g)	A	0.0	100.0	0.6±0.04	76.9	4.7±2.43	34,7
9. NFm (1.0 l/g) + Inshur Perform, KS	L	1.2±0.80	14.3	2.0±0.46	47.4	7.6±2.59	0,0
(0.2 l/g)	A	1.6±0.57	0.0	1.1±0.18	57.7	4.8±1.07	33,3
10. Vitaplan, SP (20 g/t)	L	1.9±1.48	0.0	5.5±1.53	0.0	7.8±2.23	0,0
	A	0.7±0.54	36.4	1.8±0.76	30.8	5.0±0.67	30,6
11. NKteoa (1.0 l/t) + Vitaplan, SP	L	2.7±2.01	0.0	3.2±0.92	15.8	9.0±1.55	0,0
(20 g/t)	A	0.0	100.0	1.2±0.38	53.8	7.4±1.96	0,0
12. NFm (1.0 l/t) + Vitaplan, SP	L	2.1±0.82	0.0	3.7±1.21	2.6	15.9±8.10	0,0
(20 g/t)	A	1.1±0.74	0.0	0.9±0.51	65.4	7.6±3.19	0,0
LSD_{05} (cultivar)		0,49		0.63		1.92	
LSD_{05} (preparations)		1,20		1.41		4.90	
LSD_{05} (cultivar, preparations)		1,74		2.03		7.36	

N o t e. L — Leningradsky, A — Ataman; R — disease development rate, BE — biological effectiveness. Each sample consists of 10 grains. For detailed descriptions, see the Materials and methods section.

The protective effect of seed treatment with nano-compositions was clearly expressed in the Ataman variety and, depending on the phase of development of the culture, amounted to 15.3-57.7% for the NKteoa silica-ash composition, 15.3-36.4% for NFm. An unstable, predominantly higher protective effect was noted when the biological preparation was used together with nanocomposites compared to the variant when the treatment was carried out only with the biological preparation (Table 2). However, the treatments with a biological product were less effective than the options for the combined use of nanocomposites with the fungicide Inshur Perform, KS.

The treatment of vegetative barley plants with NFm and NFtr nanocompositions turned out to be ineffective in relation to helminthosporium leaf spots: 15.6 and 21.6%for the Leningradsky variety, respectively, 20.3 and 42.2% for the Ataman variety. A weak protective effect occurred when plants were treated with microfertilizer KKhM-G, and a consistently high effect was observed in those variants where the use of microfertilizer and the above nanocompositions was supplemented by fungicidal treatment with Zantara, CE (Table 3). The weak development of powdery mildew (1.1 and 1.6%) and rhynchosporiosis (0.1 and 1.4%), observed on both varieties of spring barley in 2017, did not allow us to reliably assess the effectiveness of the studied compositions and preparations on infestation. leaf apparatus of plants with these diseases.

3. Efficiency of plant treatment with nanocompounds, microfertilizer, chemical fungicide and their combinations against helminthosporium leaf spots in spring barley (*Hordeum vulgare* L.) varieties at different stages of development (n = 4, M±SEM; Leningrad Province, 2017)

Variant	Cultivar	Stage: grain filling, R, %	grain filling, BE, %	milky ripe, R, %	milky ripe, BE, %
1. Control (water)	L	8.0±0.57		37,9±6,58	
	A	11.6±0.88		12,8±1,98	
2. Zantara, CE (0.8 l/ha)	L	4.9±1.79	38,8	11,7±1,11	69,1
	A	2.5±0.76	78,4	3,4±0,49	73,4
3. Zantara, CE (0.4 l/ha)	L	6.2±1.49	22,5	14,9±2,71	60,7
	A	3.2±0.40	72,4	4,8±0,95	62,5
4. KKhM-G (3.0 l/ha)	L	6.8±0.62	15,0	33,1±4,19	12,7
	A	12.7±1.61	0,0	8,8±1,96	31,3
5. NFm (1.0 l/ha)	L	6.5±0.73	18,8	32,0±1,30	15,6
	A	11.9±1.25	0,0	10,2±0,54	20,3
6. NFtr (1.0 l/ha)	L	7.5±1.25	6,3	29,7±4,53	21,6
	A	12.5±1.10	0,0	7,4±0,28	42,2
7. KKhM-G (3.0 l/ha) + Zantara, CE (0.8 l/ha)	L	4.8±1.19	40,0	12,4±1,52	67,3
	A	1.9±0.31	83,6	3,2±0,40	75,0
8. KKhM-G (3.0 l/ha) + Zantara, CE (0.4 l/ha)	L	5.5±0.82	31,3	15,3±1,90	59,6
	A	2.2±0.33	81,0	4,2±0,67	67,2
9. NFm (1.0 l/ha) + Zantara, CE (0.8 l/ha)	L	4.1±0.92	48,8	9,0±0,82	76,3
	A	2.5±0.60	78,4	4,4±1,62	65,6
10. NFm (1.0 l/ha) + Zantara, CE (0.4 l/ha)	L	5.6±2.92	30,0	14,4±2,05	62,0
	A	2.3±0.58	80,2	4,8±0,61	62,5
11. NFtr (1.0 l/ha) + Zantara, CE (0.8 l/ha)	L	4.4±1.11	45,0	10,3±0,87	72,8
	A	1.8±0.10	84,5	3,3±0,21	74,2
12. NFtr (1.0 l/ha) + Zantara, CE (0.4 l/ha)	L	4.4±0.62	45,0	12,6±1,53	66,8
	A	2.2±0.27	81,0	3,6±0,20	71,9
LSD_{05} (cultivar)		1,27		2,41	
LSD_{05} (preparations)		2,68		5,45	
LSD_{05} (cultivar, preparations)		2,78		5,56	

N o t e. L — Leningradsky, A — Ataman; R — disease development rate, BE — biological effectiveness. Each sample consists of 10 grains. For detailed descriptions, see the Materials and methods section.

An analysis of the elements of the structure of the spring barley yield showed that the treatment of the seed with the studied preparations had the strongest effect on the density of the productive stalk. In Leningradsky variety, the number of productive stems increased by 11% with chemicals, by 5-12% with nanocompositions, by 8-17% with their combinations, and by 10-15% with the biofungicide and its mixtures with nanocompositions.

The yield of variety Leningradsky in the control (untreated seeds) was 38.4 c/ha. Seed dressing increased the yield by 5-18% (Fig. 2). The greatest economic effect was observed when the seed material was treated with combinations of NKteoa and a chemical fungicide (an increase of 14 and 18% relative to the control at fungicide consumption rates of 0.2 and 0.4 l/t, respectively), as well as NKteoa and the biological product Vitaplan, SP (by 19%). Sufficiently high yields were recorded in the variants with the use of a biological product alone (by 112%) and in combinations with the nanocomposition of NFm and a chemical disinfectant (by 110 and 112% at fungicide consumption rates of 0.2 and 0.4 l/t, respectively).

Somewhat different data were obtained on the Ataman variety. Firstly, this variety turned out to be more productive, and secondly, the greatest and statistically significant economic effect (increase in yield) on it was shown by seed treatment with a chemical preparation (112% vs. control) and a combination of NFm and a chemical fungicide at a dose of 0.4 l/t (116% vs. control). It should be noted that there is no economic effect in the treatment of seeds only with nanocompositions.

Foliar treatment of vegetative plants with NFm and NFtr nanocompositions did not increase the yield of spring barley cv. Leningradsky. The use of microfertilizers KKhM-G followed by the use of fungicides gave a high economic effect. In this case, an excess of the individual effect of the microfertilizer or fungicide was observed. The same was noted for plant treatment of with nano-compounds and the fungicide Zantara, CE. Yields were 6% and 35% higher with NFm (0.4 l/ha) and Zantara, CE (0.8 l/ha), and 11% and 50% higher with NFtr (0.4 l/ha) and Zantara, CE (0.8 l/ha).

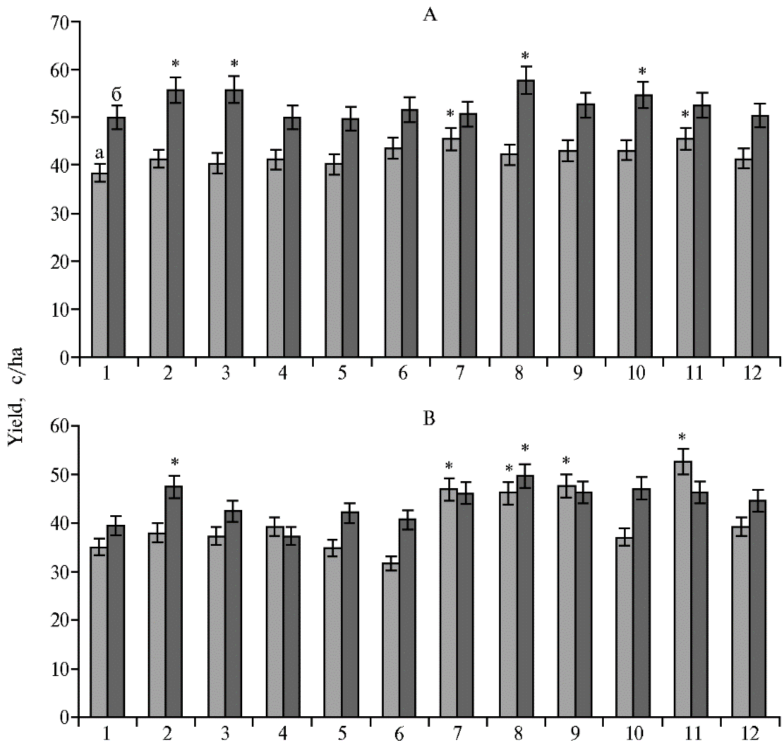

Fig. 2. Yields of spring barley (*Hordeum vulgare* L.) varieties Leningradsky (a) **and Ataman (b) after pre-sowing seed treatment** (A) **or spraying plants** (B) **with solutions of nanocompositions, KKhM-G, chemical and biological fungicides and their combinations** (n = 4, M±SEM; Leningrad Province, 2017).

A: 1 — control (water); 2 — Inshur Perform, KS (0.4 l/t); 3 — Inshur Perform, KS (0.2 l/t); 4 — silica composition NKteoa (1.0 l/t); 5 — composition of a fullerene derivative with methionine and NFm microelements (1.0 l/t); 6 — NKteoa (1.0 l/t) + Inshur Perform, KS (0.4 l/t); 7 — NKteoa (1.0 l/t) + Inshur Perform, KS (0.2 l/t); 8 — NFm (1.0 l/t) + Inshur Perform, KS (0.4 l/t); 9 — NFm (1.0 l/t) + Inshur Perform, KS (0.2 l/t); 10 — Vitaplan, SP (20 g/t); 11 — NKteoa (1.0 l/t) + Vitaplan, SP (20 g/t); 12 — NFm (1.0 l/t) + Vitaplan, SP (20 g/t).

B: 1 — control (water); 2 — Zantara, CE (0.8 l/ha); 3 — Zantara, CE (0.4 l/ha); 4 — silicon-containing chelate microfertilizer KKhM-G (3.0 l/ha); 5 — NFm (1.0 l/ha); 6 — composition of fullerene derivative with threonine and microelements NFtr (1.0 l/ha); 7 — KKhM-G (3.0 l/ha) + Zantara, CE (0.8 l/ha); 8 — KKhM-G (3.0 l/ha) + Zantara, EC (0.4 l/ha); 9 — NFm (1.0 l/ha) + Zantara, CE (0.8 l/ha); 10 — NFm (1.0 l/ha) + Zantara, CE (0.4 l/ha); 11 — NFtr (1.0 l/ha) + Zantara, CE (0.8 l/ha); 12 — NFtr (1.0 l/ha) + Zantara, EC (0.4 l/ha).

Each sample was harvested from a 1 m^2 area. For detailed description, see the Materials and Methods section.

The economic effect of a single treatment of cv. Ataman with the fungicide Zantara, CE, depending on the rate of application of the drug (0.4 and 0.8 l/ha), was 8 and 20%, respectively, The treatment with KKhM-G microfertilizer and the fungicide resulted in 17 and 26% effects, with NFm and the fungicide in 17 and 19% effects, with NFtr and the fungicide in 13 and 17% effects, Thereof, the efficiency of half-dose fungicide significantly increased due to the developed silicon-containing microfertilizers KKhM-G and nanocompositions. A lower economic effect was noted in the variants where the use of only KKhM-G microfertilizer or carbon nanocomposites was envisaged.

The most objective indicator in assessing the effect of treating vegetative plants with fungicides is the 1000-grain weight. According to the obtained values, in Leningradsky variety, the combined use of KKhM-G microfertilizer and the fungicide at a dose of 0.4 and 0.8 l/ha increased this indicator by 9 and 17% vs. control. According to the effect on the 1000-grain weight in Ataman variety, foliar treatments with a fungicide were distinguished (an increase vs. control by 110 and 112% at application rates of 0.4 and 0.8 l/ha, respectively) and combined application of NFm with a fungicide (by 111 and 112%).

According to data for 2017, the most effective treatment options for protecting spring barley from diseases were identified, which in 2018 were included in the study of the effectiveness of the technological scheme for the use of nanocompositions. To enhance the protective effect during seed treatment, 0.1% titanium dioxide in the form of anatase was added to the composition of NKteoa in addition to the charge of detonation nanodiamond [22].

According to phytoexpertise, the treatment of seeds of spring barley Leningradsky variety with a silica-sol nanocomposition led to a decrease in infection with *Fusarium* fungi by 43.7% and *Alternaria* blight by 23.5%, but had no effect on the main causative agent of root rot, the fungus *C. sativus* (Table 4).

4. Efficiency of seed treatment with the nanocomposition, chemical fungicide and their combinations against seed infection in spring barley (*Hordeum vulgare* L.) cv. Leningradsky (n = 10, M±SEM; Leningrad Province, 2018)

Variant	Microbiota					
	pathogenic				saprotrophic	
	Cochliobolus sativus		*Fusarium* spp.		*Alternaria* spp.	
	DI, %	BE, %	DI, %	BE, %	DI, %	BE, %
1. Control (water)	6.0±1.35		16.0±2.05		81.0±5.41	
2. Inshur Perform, KS (0.4 l/t)	4.0±1.08	33.3	13.0±1.96	18.7	39.0±4.87	51.9
3. Inshur Perform, KS (0.2 l/t)	3.0±0.88	50.0	18.0±2.25	0.0	45.0±4.98	44.4
4. NKteoa (1.0 l/t)	17.0±2.31	0.0	9.0±1.87	43.7	62.0±5.14	23.5
5. NKteoa (1.0 l/t) + Inshur Perform, KS (0.4 l/t)	13.0±1.45	0.0	13.0±2.41	18.7	53.0±4.52	34.6
6. NKteoa (1.0 l/t) + Inshur Perform, KS (0.2 l/t)	8.0±2.06	0.0	14.0±2.87	12.5	71.0±6.42	12.3
LSD_{05}	1.51		3.53		12.46	

Note. DI — disease incidence, BE — biological effectiveness. Each sample consists of 10 grains. For detailed descriptions, see the Materials and methods section.

Under severe damage by root rot in 2018 caused by a long dry period during initial crop growth, there was a weak and short effect of seed treatment with the chemical fungicide Inshur Perform, KS on the disease incidence (Table 5). With the combined seed treatment with the NKteoa nanocomposition and a chemical fungicide, the effectiveness of protection against root rot increased to 10.1-21.5% vs. the control, but only at the full rate of Inshur Perform, KS. An even stronger effect (decrease in the development of root rot by 13.9-35.4% relative to the control) was observed in the variant with the treatment of seeds with a silica nanocomposition and a chemical preparation at the full rate of application, followed by a 3-fold treatment of vegetative plants with NFtr.

In 2018, a significant protective effect of seed treatment with fungicides was manifested in relation to Helminthosporium leaf spots (Table 6). In variants with inoculum dressing with Inshur Perform, KS, the development of helminthosporiasis, depending on the dose of application, decreased by 20.0-32.2% (0.4 l/t) and 17.5-32.9% (0.2 l/t) vs. control. With pre-sowing treatment with silica nanocomposition, the disease incidence rate decreased by 7.5-15.4%.

5. Efficiency of seed and plant treatments with nanocompounds, chemical fungicide and their combinations against root rot in spring barley (*Hordeum vulgare* L.) cv. Laningradsky varieties at different stages of plant development (n = 4, M±SEM; Leningrad Province, 2018)

Variant	Stage					
	tillering		stem extention		heading	
	R, %	BE, %	R, %	BE, %	R, %	BE, %
1. Control (water)	30.2±3.35		34,0±2,68		44,5±8,28	
2. Inshur Perform, KS (seeds 0.4 l/t) + water (plants 300 l/ha)	25.5±4.07	15,6	28,8±1,99	15,3	40,0±3,81	10,1
3. Inshur Perform, KS (seeds 0.2 l/t) + water (plants 300 l/ha)	30.4±6.85	0,0	32,0±4,32	5,9	42,2±4,87	5,2
4. NKteoa (seeds 1.0 l/t) + water (plants 300 l/ha)	29.4±1.83	2,6	33,6±9,09	1,2	45,0±8,11	0,0
5. NKteoa (seeds 1.0 l/t) + Inshur Perform, KS (seeds 0.4 l/t) + water (plants 300 l/ha)	24.2±6.27	19,9	26,7±7,66	21,5	40,0±3,81	10,1
6. NKteoa (seeds 1.0 l/t) + Inshur Perform, KS (seeds 0.2 l/t) + water (plants 300 l/ha)	30.8±3.49	0,0	34,3±7,04	0,0	39,8±8,67	10,6
7. Water (seeds) + NFtr (plants 1.0 l/ha)	38.5±8.15	0,0	43,9±6,51	0,0	53,5±5,93	0,0
8. Inshur Perform, KS (seeds 0.4 l/t) + NFtr (plants 1.0 l/ha)	25.0±4.45	17,2	28,7±2,68	15,6	39,3±2,25	11,7
9. Inshur Perform, KS (seeds 0.2 l/t) + NFtr (plants 1.0 l/ha)	29.5±8.05	2,3	29,2±2,89	14,1	44,9±2,78	0,0
10. NKteoa (seeds 1.0 l/t) + NFtr (plants 1.0 l/ha)	24.3±4.31	19,5	39,0±5,54	0,0	48,8±4,53	0,0
11. NKteoa (seeds 1.0 l/t) + Inshur Perform, KS (seeds 0.4 l/t) + NFtr (plants 1.0 l/ha)	19.5±3.68	35,4	22,2±8,16	34,7	38,3±7,64	13,9
12. NKteoa (seeds 1.0 l/t) + Inshur Perform, KS (seeds 0.2 l/t) + NFtr (plants 1.0 l/ha)	31.2±5.58	0,0	34,4±7,32	0,0	46,0±4,26	0,0
LSD_{05}	13.61		14,49		16,94	

N o t e. R — disease development rate, BE — biological effectiveness. Each sample consists of 30 plants. For detailed descriptions, see the Materials and methods section.

6. Efficiency of seed and plant treatment with nanocompounds, chemical fungicide and their combinations against helminthosporium leaf spots in spring barley (*Hordeum vulgare* L.) cv. Leningradsky at different stages of development (n = 4, M±SEM; Leningrad Province, 2018)

Variant	Stage			
	grain filling		milky ripe	
	R, %	BE, %	R, %	BE, %
1. Control (water)	4.0±0.34		14.3±2.42	
2. Inshur Perform, KS (seeds 0.4 l/t) + water (plants 300 l/ha)	3.2±0.16	20.0	9.7±0.33	32.2
3. Inshur Perform, KS (seeds 0.2 l/t) + water (plants 300 l/ha)	3.3±0.19	17.5	9.6±1.45	32.9
4. NKteoa (seeds 1.0 l/t) + water (plants 300 l/ha)	3.7±0.38	7.5	12.1±1.77	15.4
5. NKteoa (seeds 1.0 l/t) + Inshur Perform, KS (seeds 0.4 l/t) + water (plants 300 l/ha)	2.8±0.25	30.0	11.0±1.08	23.1
6. NKteoa (seeds 1.0 l/t) + Inshur Perform, KS (seeds 0.2 l/t) + water (plants 300 l/ha)	3.2±0.24	20.0	13.2±0.86	7.7
7. Water (seeds) + NFtr (plants 1.0 l/ha)	3.6±0.16	10.0	12.5±0.72	12.6
8. Inshur Perform, KS (seeds 0.4 l/t) + NFtr (plants 1.0 l/ha)	2.9±0.35	27.5	8.5±1.02	40.6
9. Inshur Perform, KS (seeds 0.2 l/t) + NFtr (plants 1.0 l/ha)	3.2±0.67	20.0	10.0±1.39	30.1
10. NKteoa (seeds 1.0 l/t) + NFtr (plants 1.0 l/ha)	2.8±0.37	30.0	11.5±1.41	19.6
11. NKteoa (seeds 1.0 l/t) + Inshur Perform, KS (seeds 0.4 l/t) + NFtr (plants 1.0 l/ha)	2.9±0.23	27.5	9.9±0.35	30.8
12. NKteoa (seeds 1.0 l/t) + Inshur Perform, KS (seeds 0.2 l/t) + NFtr (plants 1.0 l/ha)	3.7±0.48	7.5	12.1±0.62	15.4
LSD_{05}	0.94		3.40	

N o t e. R — disease development rate, BE — biological effectiveness. Each sample consists of 30 plants. For detailed descriptions, see the Materials and methods section.

The icidence of helminthosporiosis decreased by 10.0-12.6% vs. control unde foliar treatment with NFtr nanocomposition and by 19.6-30.0% under seed treatment with silica-sol nanocomposition (see Table 6). A positive effect of NFtr treatment was also noted complementing the effect of seed dressing. Seed treatment with Inshur Perform, KS and 3-fold plant treatment with the NFtr nanocomposition decreased the helminthosporiosis incidence by 27.5-40.6% vs. control at 100% application rate and by 20.0-30.1% at 50% application rate.

The high productivity of spring barley plants was typical for the variants with the treatment of seeds, as well as seeds and vegetative plants with the tested nanocompositions. Exceeding the control by the mass of grain per ear in both of these options was 9%. According to the density of the productive stem stand, a variant was distinguished (15% higher than the values in the control), which provided for the sequential treatment of seeds with a silica-sol nanocomposition and the chemical preparation Inshur Perform, KS (0.4 l/t) with a further 3-fold treatment of vegetative plants with a nanocomposition of NPtr.

Analyzing the yields obtained, we can conclude that there are no significant differences between the control and the studied options for the treatment of seeds and vegetative plants of spring barley (Fig. 3).

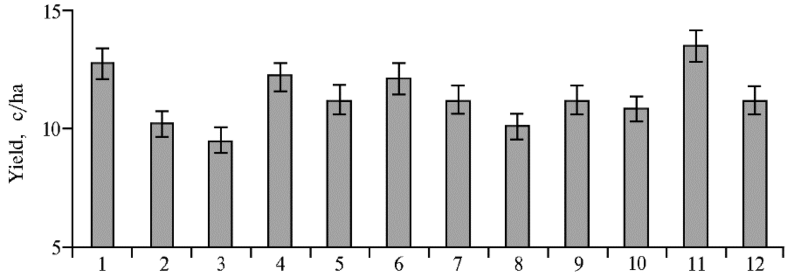

Fig. 3. Yield of spring barley (*Hordeum vulgare* L.) cv. Leningradsky after pre-sowing seed treatment and spraying plants with nanocompositions, chemical fungicide and their combinations: 1 — control (water), 2 — Inshur Perform, KS (seeds 0.4 l/t) + water (plants 300 l/ha), 3 — Inshur Perform, KS (seeds 0.2 l/t) + water (plants 300 l/ha), 4 — NKteoa (seeds 1.0 l/t) + water (plants 300 l/ha), 5 — NKteoa (seeds 1.0 l/t) + Inshur Perform, KS (seeds 0.4 l/t) + water (plants 300 l/ha), 6 — NKteoa (seeds 1.0 l/t) + Inshur Perform, KS (seeds 0.2 l/t) + water (plants 300 l/ha), 7 — water (seeds) + NFtr (plants 1.0 l/ha), 8 — Inshur Perform, KS (seeds 0.4 l/t) + NFtr (plants 1.0 l/ha), 9 — Inshur Perform, KS (seeds 0.2 l/t) + NFtr (plants 1.0 l/ha), 10 — NKteoa (seeds 1,0 l/t) + NFtr (plants 1.0 l/ha), 11 — NKteoa (seeds 1.0 l/t) + Inshur Perform, KS (seeds 0.4 l/t) + NFtr (plants 1.0 l/ha), 12 — NKteoa (seeds 1.0 l/t) + Inshur Perform, KS (seeds 0.2 l/t) + NFtr (plants 1.0 л/га) (n = 4, M±SEM; Leningrad Province, 2018). Each sample was harvested from a 1 m^2 area. For detailed description, see the Materials and Methods section.

Initially, carbon and silica nanocomposites were considered as plant growth regulators, and their use in agriculture was aimed at increasing the quantity and quality of the crop, achieved by increasing field germination, photosynthesis intensity, and the production process [35-37]. Also, their ability to increase the resistance of plants to stressors of a biotic and abiotic nature, including the action of harmful organisms, was noted [38, 39]. The results of our studies generally indicate weak and in many cases statistically unreliable effects of treating seeds and vegetative plants with carbon and silica-ash nanocompositions to protect spring barley from root rot and leaf diseases. This can be explained by their directed action not on the pathogen attacking plant cells, but on the activation of plant metabolism and immunity, which is confirmed by the actual data of other studies [40, 41]. However, in our experiments, we have revealed a pronounced biocidal effect of silica nanocomposition against seed infection, namely the genus *Fusarium* fungi. Another important conclusion concerns the great prospects for the use of carbon and silica-sol compositions on spring barley varieties with a long growing season, which are more susceptible to the influence of phytopathogens. A possible way to enhance the protective effect is the combined use of the nanocompositions and chemicals designed to protect cultivated plants from phytopathogens. Due to the pronounced additive effect, the possibility of a significant reduction in the application of fungicides opens up, which was proved by the example of the combined use of biological and chemical preparations in the protection of spring barley from diseases [32].

The data obtained by us indicate certain prospects for the use of new carbon and silica-sol nanocompositions in the protection of spring barley from diseases in order to reduce the pesticide load during the cultivation of this crop in the North-West Russia. The co-application of chemical or biological fungicides with nanocompositions is highly effective due to enhanced protective effects and a potentially reduced dosage of chemicals and biologicals. It is necessary to continue research for scientifically based selection of the most effective combinations of nanocompositions and pesticides and improvement of technological schemes for their application.

Thus, the nanocompositions used in pure form have a weak and unreliable effect on the development of root rot on the early-ripening barley variety Leningradsky (0-5.3%), The effect on the mid-late variety Ataman is more pronounced and stable (15.3-57.7 %, $p < 0.05$). Pre-sowing seed treatment with a silica-sol nanocomposition based on 1 wt.% tetraethoxysilane (pH 2-3) with macro- and microelements and 0.1% titanium dioxide results in a decrease in the development of helminthosporium leaf spots by 7.5-15.4% ($p < 0.05$). The most prolonged and pronounced protective effect against root rots occurs under a combined treatment of seeds with a silica-sol nanocomposition and the chemical fungicide Inshur Perform, KS, followed by foliar treatment with a nanocomposition based on derivative of C_{60} fullerene with threonine. To protect spring barley from *Helminthosporium* blotches, the studied nanocompositions in their pure form were ineffective. The symptoms on the two upper leaves decreased by 16-22 and 20-42% in Leningradsky and Ataman varieties, respectively ($p < 0.05$). When seeds are treated with the silica-sol composition and vegetative plants with the nanocomposition based on the C_{60} fullerene derivative with threonine, the development of helminthosporiasis decreases by 19.6-30.0% ($p < 0.05$) which is equivalent to the treatment of seeds and plants with the chemical preparation Inshur Perform, KC at half the rate of use. Two treatments, a combined 3-fold application of the NFtr nanocomposition with a single application of the chemical fungicide Zantara, KE and the use of microfertilizer KKhM-G with the chemical fungicide Zantara, KE, provide high protective effect on both varieties. The best protocol to protect spring barley plants from root and leaf diseases compeises the combined seed treatment with a silica-sol nanocomposition and the chemical fungicide Inshur Perform, KS together with a 3-fold treatment of vegetative plants with a nanocomposition based on the threonine derivative of C_{60} fullerene and a single treatment with chemical fungicide Zantara, KE. Reducing the rate of use of a chemical preparation leads to a significant loss of efficiency and a reduction in the protective period and, thereof, is advisable only if weak manifestation of diseases is expected. High biological and economic efficiency, comparable to fungicidal treatments at 100% application rate, was ensured by the combined use of KKhM-G microfertilizer with a fungicide (50% application rate) and the combined use of a nanocomposition based on the methionine derivative of C_{60} fullerene and a fungicide (50% application rate).

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THE FORMATION OF PRODUCTIVITY OF GRAIN CROPS WITH INTRODUCING HYDROGELS UNDER MODEL SOIL DROUGHT AND IN FIELD CONDITIONS

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Abstract

The use of water-absorbing hydrogels capable to regulate the soil water regime allows for a significant increase in crop production in arid and semi-arid climatic zones. Polyacrylamide and polyacrylonitrile hydrogels cyclically (over several years) absorb and release moisture, so they are most effective in agriculture. This paper shows that three polymer gels of different origin have similar effect on the yield structure and productivity of grain crops compared under controlled soil drought and in field tests. Domestic gels had the greatest effect on the 1000-grain weight. The type of hydrogel (either sodium or potassium base) did not significantly influenced the yield structure parameters. This work aimed to evaluate grain crops' productivity and yield structure as affected by polymer gels V-415 K and Ritin-10 (Russia) under simulated soil drought compared to the polymer Aquasorb (France) under field conditions of a zone of insufficient moisture. Microfield trials were performed on spring barley (*Hordeum vulgare* L.) cv. Leningradsky in 2015, spring wheat (*Triticum aestivum* L.) cv. Daria in 2016 and spring barley cv. Ataman using bottomless pots (Agrophysical Institute, Menkovsky branch, Leningrad Province) of a 0.075 m^2 area and a 0.0025 m^3 volume. The pots were filled with sod-podzolic sandy loamy soil according to the soil horizons' order. The treatments were a control ($N_{90}P_{90}K_{90}$); $N_{90}P_{90}K_{90}$ + Ritin-10 at 10-12 cm depth; $N_{90}P_{90}K_{90}$ + V-415 K at 10-12 cm depth; $N_{90}P_{90}K_{90}$ + Ritin-10 at 20-22 cm depth; $N_{90}P_{90}K_{90}$ + V-415 K at 20-22 cm depth. The dose of each hydrogel was 4 g/m^2, the seeding rate was 50 pcs/pot. Soil moisture in the pots was measured twice a week to calculate necessary watering rate. The effect of soil drought (55-60 % water holding capacity) was assessed from the tillering phase to full ripeness. The productivity of winter wheat (*T. aestivum*) cv. Steklovidnaya 24 as influenced by polymer gel Aquasorb (SNF s.a.s., France) was studied in the Republic of Kazakhstan in 2015-2017 (experimental fields of the Kazakh Research Institute of Agriculture and Plant Growing). Two doses of the absorbent (20 and 40 kg/ha) and their combination with nitrogen supplementation (N_{45}) were tested. The total number of plants per pot (per 1 m^2 in field trials), the number of productive plants, and productive bushiness coefficient, the ear length, the number of grains per ear, the grain mass per ear, and the 1000-grain weight were determined. The grain yield under a controlled "drought" when the hydrogels were introduced into the root layer (10-12 cm) differed slightly from the control (an increase by only 3-4 %). For the 20-22 cm depth, the yield exceeded the control by 25.0-27.7 % ($p < 0.01$). The hydrogels significantly influenced the yield structure parameters for productive bushiness coefficient, the number of grains per ear and the 1000-grain weight. With Ritin-10 hydrogel, the yield inversely correlated with the number of productive stems ($r = -0.83$), the number of grains per ear ($r = -0.78$) and the grain weight per ear ($r = -0.78$). With V-415 K, the correlation coefficients showed a close relationship between yield and tillering ($r = 0.70$), with the grain mass per ear ($r = 0.74$) and with the 1000-grain weight ($r = 0.71$). Under the simulated soil drought, the hydrogels had the greatest impact on the 1000-grain weight. Under field conditions of Kazakhstan, the yield of winter wheat largely depended on weather conditions. In a dry 2015, the hydrogel at a dose of 40 kg/ha with nitrogen fertilizers increased the crop yield by 6.6 c/ha compared to the control. The hydrogel together with nitrogen fertilizers also significantly ($p < 0.05$) increased the crop yield in a moderately wet 2016; in a wet 2017, the grain yield increased significantly ($p < 0.01$), up to 16.4-23.8% depending on the dose of the hydrogel. Aqusorb gel significantly affected all elements of yield structure. In the semi-arid period, when 20 kg/ha of Aquasorb hydrogel was applied, there was an inverse correlation between the yield and the grain mass per ear ($r = -0.99$) and the 1000-grain weight ($r = -0.98$). For a dosage of 40 kg/ha, there was a close correlation with the number of grains per ear ($r = -0.99$) and the grain mass per ear ($r = -0.87$). Wheat yield also had a close inverse relationship with the number of grains per ear ($r = -0.83$) when Aquasorb (20 or 40 kg/ha) was used with nitrogen fertilizers. In humid and moderately humid years, the dependence of yield on yield structure indicators is also strong ($r = 0.84$-0.99). Thus, the hydrogel introduced into the 10-12 cm soil layer dries out without watering and does not act as a water-retaining soil additive. A significant increase in the grain yield can be obtained by laying polymer gels to a depth of 20-22 cm after water-charging irrigation of the arable layer. In field conditions, during dry growing seasons, it is necessary to apply a high dose of hydrogel (40 kg/ha) in combination with nitrogen fertilizers. In moderately humid and humid growing seasons, a dose of 20 kg/ha is sufficient in combination with nitrogen fertilization.

Keywords: polymer gel, soil drought, water stress, barley, spring wheat, winter wheat, yield

Humidity is a limiting factor in growing crops. Plants that lack moisture lag behind in development, are more susceptible to diseases and pests, and do not compete well with weeds, which affects yields [1-3].

Moisture-absorbing polymer gels are used in various sectors of the national economy. In crop production and agriculture, they allow you to regulate the water regime of soils in arid and semi-arid climatic zones. Due to the network structure of the macromolecule, the hydrogel can accumulate a large amount of water in its volume [4, 5]. Water-absorbing polymer gels sorb melt or rain water, and in case of drought slowly release moisture (that is, they use the condensate of subsoil water vapor), nourishing the plants. Depending on the swelling capacity of the hydrogel, the amount of absorbed water can increase by 1000-1500% [6]. During swelling, there is no strong binding of water to the polymer molecule, so water remains available for plants [7].

Polyacrylamide and polyacrylonitrile hydrogels have the ability to cyclically (over several years) absorb and release moisture, so their use is most effective in agricultural activities [8]. Biopolymers are especially attractive for agriculture because they are biodegradable [9]. Some bacteria produce enzymes capable of converting biopolymers into water, carbon dioxide, methane, and biomass [9, 10].

Nutrients necessary for good plant growth (macronutrients N, P, K, Ca, Mg, S, micronutrients B, Cl, Co, Cu, Fe, Mn, Mo, Ni, Zn) are often not available in sufficient quantities in the environment. Polymeric materials can be used to deliver agrochemicals to the soil without causing pollution [11-14]. It has been shown that it is best to introduce nutrients into the polymer in the form of complex compounds (chelates), which are difficult to decompose into ions and cannot affect the decrease in the water absorption capacity of the polymer gel [15, 16]. By reducing losses due to gravity and physical evaporation, the hydrophilic polymer retains an additional supply of moisture in the soil profile [17, 18]. Hydrogels improve soil agrophysical properties [18, 19].

Plants, both in their natural environment and during cultivation, are often subjected to environmental stress [8, 20]. Water-absorbing polymer gels are very effective in conditions of elevated temperature and soil moisture deficiency [20-22]. The lack or excess of heat or moisture during certain periods of ontogenesis significantly affects the growth and development of plants, as well as the yield and quality of grain. When hydrogels are introduced into the root-inhabited soil layer, an additional supply of moisture is created, which is necessary during critical periods of development (tillering – stem elongation). L.O. Ekebafe et al. [23] and G. Cheruiyot et al. [24] confirmed that polymers help maintain soil moisture by changing the distribution of soil particles, liquid and gas phases when water is added, which increases the proportion of liquid compared to gas.

A high percentage of moisture retention when using a hydrogel during the period of active growth contributes to a high intensity of photosynthesis [9, 25]. In addition, by reducing water stress throughout the growing cycle, the use of polymers improves crop quality [20, 22, 26]. R. Hayat et al. [27] found that the amount of water absorbed by the polymer affected yield parameters. The lack of moisture in the soil during the period of grain filling significantly affects the structure of the crop and the overall productivity of plants. Adding a moisture-swellable polymer to the soil increased the 1000-grain weight in corn and soybeans [28] and the yield in corn and winter wheat [23, 29]. J. Grabinski et al. [30] note that the presence of hydrogel in the soil has an insignificant effect on the number of plants, but the effect on the number of grains per ear and the 1000-grain weight was more significant. The 1000-grain weight is also affected by meteorological factors and agricultural practices [20, 27, 29].

Many arid and semi-arid regions face problems with uncertain and insufficient rainfall [8, 23, 31, 32]. In arid climatic zones, the use of polymer gels in sandy soil (macroporous medium) seems to be one of the most significant methods for increasing its water-retaining capacity and increasing crop productivity. Hydrogel particles can serve as miniature "reservoirs" of water in the soil. Water will flow from them at the request of the root hairs through the osmotic pressure difference. Hydrogels provide the retention and release of nutrients as they are absorbed. Consequently, the plant can access fertilizers which improve crop growth and productivity [31, 32-35].

Studies on the effect of Sky Gel polymer gel (Imec®, SkyGel®, Mebiol Gel®, Mebiol, Inc., Japan) on the growth, yield and water loss of wheat plants in Iraq, showed that 4, 8 and 12% Sky Gel added to the soil ncreased in the number of grains per spike to 48.94, 50.03 and 51.93 vs. 45.26 in the control. The 1000-grain weight increased when the gel amount increased. The highest value (4.11 g) occurred with 12% gel, the lowest (3.43 g) in the control. When 12% Sky Gel was applied, the maximum plant height (87.33 cm), root length (24.58 cm), root shoot coefficient (28.15), yield (4.83 t/ha) and the highest efficiency of water use (1.516 kg/m^3) were noted [36].

Hydrogel Ritin-10 (sodium base) (OOO RITEK-ENPC, Elektrogorsk, Russia) is a cross-linked copolymer of polyacrylamide [3, 18]. Studies conducted in the zone of unstable moisture in the conditions of the Central Caucasus showed that the introduction of this hydrogel into the root layer (10-12 cm) gives a positive effect only in years with sufficient soil moisture. In dry years, it is preferable to apply the hydrogel to a depth of 20-22 cm [2]. Hydrogel V-415 K (potassium base) (ZAO Biokatalysis, Saratov, Russia) is a cross-linked copolymer of acrylic acid acrylamide [3, 18].

This paper shows that Russian-made hydrogels are as effective as foreign polymer gels in terms of their effect on productivity and structural elements of grain crops under model soil drought. The greatest effect of domestic gels had on the 1000-grain weight. The type of hydrogel (with sodium or potassium base) did not significantly affect the crop structure.

The purpose of the work is to evaluate the effect of Russian polymer gels V-415 K and Ritin-10 on the productivity and structure of the crop yield under simulated soil drought in comparison with the foreign polymer Aquasorb which was used in the field conditions of a zone of insufficient moisture.

Materials and methods. The microfield vegetation experiment was carried out on spring barley (*Hordeum vulgare* L.) cv. Leningradsky in 2015, spring wheat

(*Triticum aestivum* L.) cv. Daria in 2016, and spring barley cv. Ataman in 2017 in a “dryer” installation (Agrophysical Research Institute, Menkovsky branch, Leningrad Province). The dryer consists of a metal frame (frame) with installed light-transmitting polycarbonate, which allows simulation of the moisture supply of the experimental site by excluding the impact of external precipitation. To avoid the influence of atmospheric precipitation, a ditch was laid around the dryer (30-35 cm width, 60-70 cm depth). The depth of the dryer is 2 m, the total area is 50 m^2, 15 m^2 were used in the experiment. To isolate the roots from groundwater, two layers of a plastic film was laid on the bottom. Atmospheric and soil droughts are modeled in arid areas during dry periods of vegetation, and soil droughts during wet periods [22].

The soil in the experiment was soddy-podzolic sandy loam, sandy loam granulometrically, with sandy moraine parent rock and loamy moraine underlain. The total content of fine dust fractions and silt fractions did not exceed 6%. The soil contained 5% physical clay (particles less than 0.01%) and 95% physical sand particles. Soil density was from 1.03 to 1.07 g/cm^3, with 9.4-10.6 m^2/g specific surface, 0.54-0.55% hygroscopic moisture, 2.95% humus; the sum of absorbed bases is 6 meq/100 g of soil. The pH in the upper layers was from 4.95 to 5.03 and decreased with depth. Phosphorus and potassium contents were sufficient [18, 37].

Bottomless pots (0.075 m^2/0.0025 m^3) were filled with soil with regard to soil horizons. The experimental design was as follows: control (the basal level of $N_{90}P_{90}K_{90}$); $N_{90}P_{90}K_{90}$ + Ritin-10 (application depth 10-12 cm); $N_{90}P_{90}K_{90}$ + V-415 K (10-12 cm); $N_{90}P_{90}K_{90}$ + Ritin-10 (20-22 cm); $N_{90}P_{90}K_{90}$ + V-415 K (20-22 cm). The hydrogel (4 g/m^2) was placed at various depths in the pots, the seeding rate was 50 grains per pot, with a 5-fold repetition under systematic layout.

Soil moisture in the pots was measured with a soil moisture meter MG-44 (OOO Vetinstument, Russia) twice a week, and the irrigation rate was calculated according to the readings. At the beginning of the growing season, the soil moisture in the pots at the dryer was 70% of the lowest moisture capacity (LW). The effect of soil drought (55-60% HB) on plant growth and development was assessed from the tillering phase to full ripeness [38].

The productivity of winter wheat (*Triticum aestivum* L.) variety Steklovidnaya 24 under the influence of Aquasorb polymer gel (SNF s.a.s., France) was studied in the experimental fields of the Kazakh Research Institute of Agriculture and Plant Growing (Republic of Kazakhstan) in 2015-2017.

The soil of the experimental fields is light chestnut light loamy, coarse-silty medium loam in mechanical structure, being 39-42% physical clay, 45-51% coarse dust, and 12-17% silt. The content of carbonates was 2.7-3.6% in the upper layers and 6.5% in the carbonate horizon, pH 8.2-8.8. The sum of absorbed bases did not exceed 12 meq/100 g of soil. Calcium accounted for 80-90%, magnesium for 10-20%. The provision of the soil with readily hydrolysable nitrogen is medium, with mobile phosphorus is low, and with exchangeable potassium is medium. The sum of salts in the upper layer did not exceed 0.12%. In the upper horizon, the soil contained 2.02% humus, 0.12-0.14% gross nitrogen. Water-physical properties were characterized as follows: 2.62-2.72 g/cm^3 specific gravity, 1.23-1.35 g/cm^3 bulk density, and 50-53% porosity. The moisture content of stable wilting is 6-8% [7, 21].

Two doses of absorbent (20 and 40 kg/ha) and their combination with nitrogen supplementation (N_{45}) were tested (the control was without absorbent and nitrogen fertilizers). The repetition of the experiment is 4-fold, the placement of variants is systematic [32]. Phenological and biometric observations were done as per the methodology of field experiments [39].

The yield structure of the studied crops was determined by the sheaf selection method according to the following indicators: the total number of plants per growing vessel (in field conditions per 1 m^2), the number of productive plants, and productive tillering. Structural analysis of the ear was carried out according to the length of the ear, the number of grains in the ear, the mass of grain from one ear, and the 1000-grain weight.

Statistical data processing was performed using one-way analysis of variance (ANOVA) and correlation analysis using the Statistics 5.0 program (StatSoft, Inc., USA). Means (*M*) and standard errors of the means (±SEM) were calculated. Differences were considered statistically significant at $p \le 0.05$.

Results. In the dryer [38], the hydrogel, when introduced into the root layer (10-12 cm), had a beneficial effect only with the early emergence of seedlings of the studied crops. With the polymer placed at a depth of 20-22 cm, the soil moisture in the growing vessels corresponded to the lowest moisture capacity [3].

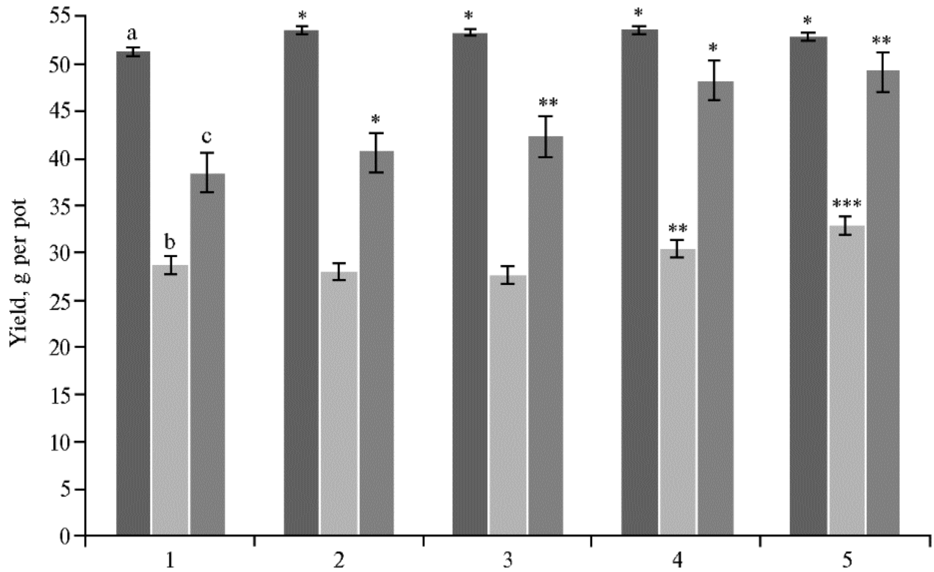

Fig. 1. Yields of spring barley (*Hordeum vulgare* L.) cv. Leningradsky (2015) (a)**, spring wheat (*Triticum aestivum* L.) cv. Darya** (2016) (b)**, spring barley cv. Ataman** (2017) (c) **under simulated soil drought as influenced by domestic polymer gels introduced into the soil at various depths:** 1 — control ($N_{90}P_{90}K_{90}$), 2 — $N_{90}P_{90}K_{90}$ + Ritin-10 (10-12 cm), 3 — $N_{90}P_{90}K_{90}$ + V-415 K (10-12 cm), 4 — $N_{90}P_{90}K_{90}$ + Ritin-10 (20-22 cm), 5 — $N_{90}P_{90}K_{90}$ + V-415 K (20-22 cm) (n = 5, *M*±SEM; test at a field "dryer" installation, Agrophysical Research Institute, Menkovsky branch, Leningrad Province).

*, **, *** Difference between the treatment and control is statistically significant at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

The use of hydrogel in the dry land in 2015 slightly increased the yield of Leningradsky barley compared to the control (by 3-4%, $p < 0.05$) (Fig. 1). The yield of cv. Daria in 2016 (see Fig. 1) was higher than the control only when both types of hydrogels were placed at a depth of 20-22 cm. For V-415 K and Ritina-10, the differences were statistically significantat at $p < 0.001$ and $p < 0.01$, respectively; Ritin-10 and V-415 K increased the yield by 5.9 and 14.6%, respectively. In 2017, the yield of barley cv. Ataman (see Fig. 1) changed slightly when gels were placed in the root zone (10-12 cm), with Ritin-10 by 5.6%, with V-415 K by 9.9 % above control. When applied to a depth of 20-22 cm, a 25.0-27.7% increase in yield was statistically significant ($p < 0.05$) for both treatments in the test (see Fig. 1, Table 1).

1. Yield components in grain crops under simulated soil drought as influenced by domestic polymer gels introduced into the soil (n = 5, M±SEM; test at a field "dryer" installation, Agrophysical Research Institute, Menkovsky branch, Leningrad Province)

Treatment	Productive stems per pot	Productive tillering	Grains per ear	Weigh, g		Yield	
				gains per ear	1000 gains	g per pot	Δ vs. control, %
			Spring barley (*Hordeum vulgare* L.) cv. Leningradsky (2015)				
Control	48±2	1.18±0.08	31±3	0.98±0.51	37.0±0.93	51.3±0.4	
Ritin-10 (10-12 cm)	53±3	1.21±0.09	30±2	1.06±0.61	36.0±0.95	53.5±0.5*	4.3
V-415 K (10-12 cm)	55±3	1.22±0.09	29±2	0.83±0.50	37.5±0.76	53.3±0.5*	3.9
Ritin-10 (20-22 cm)	56±3	1.23±0.09	33±3	1.15±0.57	38.0±0.80	53.5±0.5*	4.3
V-415 K (20-22 cm)	57±2	1.25±0.09	35±2	1.17±0.57	38.0±0.79	53.0±0.5*	3.3
			Spring wheat (*Triticum aestivum* L.) cv. Darya (2016)				
Control	48±2	1.14±0.07	24±2	0.52±0.63	29.0±1.71	28.7±0.5	
Ritin-10 (10-12 cm)	52±3	1.20±0.08	22±3	0.47±0.42	27.0±2.12	28.0±0.4	–2.4
V-415 K (10-12 cm)	54±3	1.28±0.08	27±2	0.89±0.57	33.5±1.51	27.7±0.5	–3.5
Ritin-10 (20-22 cm)	52±2	1.18±0.09	29±2	1.03±0.50	36.0±1.03	30.4±0.6**	5.9
V-415 K (20-22 cm)	56±2	1.30±0.09	28±2	0.92±0.32	34.0±1.10	32.9±0.4***	14.6
			Spring barley (*Hordeum vulgare* L.) cv. Ataman (2017)				
Control	43±2	1.10±0.08	19±3	0.83±0.22	47.0±0.66	38.5±0.6	
Ritin-10 (10-12 cm)	42±3	1.13±0.09	19±2	0.77±0.18	52.1±0.50	40.6±0.5*	5.5
V-415 K (10-12 cm)	46±2	1.12±0.09	19±2	0.82±0.21	49.0±0.56	42.3±0.6**	9.9
Ritin-10 (20-22 cm)	47±2	1.12±0.08	20±3	0.94±0.18	55.1±0.46	48.2±0.6*	25.2
V-415 K (20-22 cm)	47±2	1.12±0.09	20±3	1.04±0.17	52.0 ±0.56	49.1±0.6**	27.5

Note. $N_{90}P_{90}K_{90}$ is a basal fertilizer level. For the analysis, we partially used the data obtained by us earlier (38).
*, **, *** Difference between the treatment and control is statistically significant at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

During the active vegetation in 2015 (May-August), the average daily temperatures were slightly higher than the long-term average, only in July the air temperature was below the norm by 2.3 °C. In May, 91% of precipitation fell from the climatic norm, in June, there was 58.5% precipitation, in July 114.8% (slightly above the norm), in August — 42%. The lack of moisture was felt in almost all phases of development of spring barley (HTC = 1.5 during tillering-flowering period, HTC = 1.6 during grain filling). Only the effect of hydrogel contributed to the yield increase from 3.3 to 4.3%. In simulated soil drought, the yield components of Leningradsky variety was the best with sodium- and potassium-based hydrogels placed at a 20-22 cm depth. Productive tillering coefficient (1.23 and 1.25), the number of grains per ear (33 and 35), the weight of grain per ear (1.15 and 1.17 g) and the 1000-grain weight (38 g) exceeded the control values (see Table 1). In 2016, the average daily air temperature of the growing season slightly exceeded the norm, by 3 °C in May, by 0.8 °C in June, by 1 °C in July, and by 1.1 °C in August. Rainfall was uneven: in May there was a significant lack of precipitation, in June 116% of the norm fell, in July 131%, and in August 187%. During sowing-tillering HTC = 0.6; during the growth and development of spring wheat (tillering-flowering) HTC = 2.3. This affected the yield and indicators of the structure of the harvest of spring wheat cv. Daria. When Ritin-10 hydrogel was applied to a depth of 10-12 cm, the yield structure indicators were lower than in the control and in the variant with V-415 K. The best indicators (1000-grain weight, number of grains per spike, productive tillering) occurred at a gel application depth of 20-22 cm (see Table 1). Temperatures in May 2017 were 2.0-5.0 °C below the norm, 31% of precipitation fell from the monthly norm. The deviation of the average monthly air temperature in June from the climatic norm was 2.0-4.0 °C, the monthly precipitation amounted to 93.6%. In July, the average monthly air temperature was also 2.0-3.6 °C above the norm, the amount of precipitation coincided with the climatic norm. The average monthly temperature in August was 0.4 °C higher than the average long-term vaues, 148% of precipitation fell. The year was not very favorable for the growth and development of spring barley cv. Ataman.

When using the hydrogels Ritin-10 and V-415 K in the root zone, the parameters of the yield structure did not differ significantly from the control. Productive tillering was 1.10 in the control, and 1.12-1.13 in the variants with hydrogel. The number of grains in the spike both in the control and in the variants with hydrogel was 19 pcs. The application of hydrogels to a depth of 20-22 cm significantly increased the 1000-grain weight, 55.1 g with Ritin-10 and 52.0 g with V-415 K vs. 47.0 g in the control (see Table 1).

An analysis of the correlations between the components of yield structure (Table 2) showed that under the conditions of simulated soil drought, when Ritin-10 hydrogel was introduced into the upper root layer, the yield of grain crops closely correlated with productive tillering ($r = 0.72$), weight grains from an ear ($r = 0.62$) and 1000-grain weight ($r = 0.88$). Yield had an inverse correlation with the number of productive stems ($r = -0.64$) and a weak correlation with the number of grains per ear ($r = 0.47$). When placing the Ritin-10 gel in a layer of 20-22 cm, the correlation coefficients showed a close relationship between the yield and all elements of the crop structure. Productivity was inversely correlated with the number of productive stems ($r = -0.83$), the number of grains per ear ($r = -0.78$) and the mass of grain per ear ($r = -0.78$). When applying polymer V-415 K to a depth of 10-12 cm, a close correlation was noted between crop yields and productive tillering ($r = 0.99$), grain weight per ear ($r = 0.89$) and 1000-grain weight ($r = 0.63$); a weak relationship was established with the number of grains per ear ($r = 0.43$). When placing the B-415 K gel at a depth of 20-22 cm, a less close dependence of yield on the number of productive stems ($r = 0.58$) and grains per ear (r = 0.58) and a close relationship with productive tillering (r = 0.70), the grain weight per ear (r = 0.74) and 1000-grain weight (r = 0.71). The critical value of r at the 5% significance level is 0.63. The main elements of the crop structure, on which hydrogels had a significant effect under the conditions of simulated soil drought, are productive tillering, grain weight per ear, and 1000-grain weight.

2. Correlation coefficients (*r*) between grain yield and yield components under simulated soil drought as influenced by introduced domestic polymer gels into the soil (n = 5, M±SEM; test at a field "dryer" installation, Agrophysical Research Institute, Menkovsky branch, Leningrad Province, 2015-2017)

Productive stems	Productive tillering	Grains per ear	Grain weigh per ear	1000-grain weight
		Y = f (control)		
–0.79*	–0.80*	–0.73*	–0.68*	–0.63
		Y = f (Ritin-10, 10-12 cm layer)		
–0.64*	0.72*	0.47	0.62	0.88*
		Y = f (Ritin-10, 20-22 cm layer)		
–0.83*	0.77*	–0.55	–0.81*	0.62
		Y = f (V-415 K, 10-12 cm layer)		
0.75*	0.99*	0.43	0.89*	0.63
		Y = f (V-415 K, 20-22 cm layer		
0.58	0.70*	0.58	0.74*	0.71*

N o t e. Spring barley (*Hordeum vulgare* L.) cv. Leningradsky was grown in 2015, spring wheat (*Triticum aestivum* L.) cv. Darya in 2016, spring barley (*H. vulgare* L) cv. Ataman in 2017.
* Correlation coefficients are statistically significant at p = 0.05 (critical r value at 5% significance level is 0.63).

Therefore, under the conditions of simulated soil drought, the hydrogel placed to the soil root layer (10-12 cm) dried out and did not act as a water-retaining soil additive. The yield of grain crops increased statistically significantly ($p < 0.05$) when gels were placed at a depth of 20-22 cm. That is, in order to increase the productivity of grain crops in conditions of soil drought, it is necessary to apply hydrogel to the depth of the arable layer of 20-22 cm with preliminary moisture-charging irrigation before sowing [3, 38].

In the Republic of Kazakhstan, experiments on winter wheat cv. Steklovidnaya showed that the effect of Aquasorb hydrogel depends on meteorological conditions. The moisture supply of plants is largely determined by the amount of seasonal precipitation and the soil moisture reserves accumulated during autumn and winter. The lack of natural moisture reserves in the soil significantly reduces the yield of winter wheat [21, 29]. The southeast of Kazakhstan, where field trials were carried out, is a zone of rain-fed agriculture. During the growing season of grain crops (May-July), only 30-35% of the average annual precipitation falls there, the rest - in the post-harvest and cold periods of the year.

The grain yield in 2015 in the control was 27.0 c/ha. With Aquasorb at a dose of 20 and 40 kg/ha, the yield was 28.0 c/ha (3.7% increase) and 32.2 c/ha. (19.3%) (Fig. 2). Nitrogen fertilization contributed to an increase in yield in the control up to 27.9 c/ha. In experimental variants with hydrogel, this agrotechnical method statistically significantly ($p < 0.05$) increased the grain yield to 29.2 c/ha when applying the gel at a dose of 20 kg/ha and to 35.7 c/ha at 40 kg/ha. During the growing season of 2015, only 305 mm of precipitation fell, the year turned out to be moderately dry, so the influence of the hydrogel was undeniable. According to weather conditions, 2016 was moderately humid, which favorably affected the yield. It varied from 29.0 q/ha in the control to 32.5 q/ha (increase 12.1%) and 33.6 q/ha (15.9%) at a hydrogel dose of 20 and 40 kg/ha, respectively. Aquasorb in combination with fertilizing with nitrogen fertilizers increased the yield by 3.2 c/ha (10.0%, $p < 0.05$) and 5.4 c/ha (16.9%, $p < 0.01$), respectively, in the variants N45 + Aquasorb (20 kg/ha) and N45 + Aquasorb (40 kg/ha) (see Fig. 2).

3. Components of winter wheat (*Triticum aestivum* L.) cv. Steklovidnaya 24 yield as influenced by Aquasorb (Frnace) **polymer gel introduced into the soil** (n = 4, M±SEM; field test, zone of insufficient moisture of the Republic of Kazakhstan)

Treatment	Productive stems per pot	Productive tillering	Grains per ear	Weigh, g		Yield	
				gains per ear	1000 gains	c/ha	Δ vs. control, %
2015							
Control	345±1.3	1,72±0,2	27±1	0,98±0,10	40,4±0,4	27,0±0,60	
Aquasorb (20 kg/ha)	348±1.1	1,75±0,2	30±1	1,06±0,20	40,7±0,3	28,0±0,62	3,7
Aquasorb (40 kg/ha)	363±1.1	1,83±0,1	32±2	1,14±0,20	41,3±0,5	32,2±0,60**	19,3
Control + N_{45}	347±1.2	1,94±0,2	29±1	1,03±0,14	40,6±0,4	27,9±0,63	3,3
Aquasorb (20 kg/ha) + N_{45}	353±1.3	2,02±0,2	33±1	1,17±0,20	41,0±0,6	29,2±0,61**	8,2
Aquasorb (40 kg/ha) + N_{45}	377±1.5	2,10±0,2	35±1	1,22±0,13	41,7±0,5	35,7±0,60**	32,2
2016							
Control	353±1.2	1,83±0,2	30±1	1,04±0,12	40,9±0,4	29,0±1,07	
Aquasorb (20 kg/ha)	371±1.5	1,89±0,2	33±2	1,09±0,10	41,4±0,5	32,5±0,70*	12,1
Aquasorb (40 kg/ha)	380±1.4	1,93±0,1	34±2	1,15±0,20	41,9±0,6	33,6±0,70**	15,9
Control + N_{45}	365±1.4	1,98±0,2	33±1	1,08±0,10	41,5±0,5	31,9±1,10	10,0
Aquasorb (20 kg/ha) + N_{45}	388±1.5	2,04±0,1	34±1	1,18±0,20	42,0±0,5	35,1±0,60**	21,0
Aquasorb (40 kg/ha) + N_{45}	397±1.4	2,07±0,2	35±2	1,27±0,13	42,7±0,6	37,3±0,63*	28,6
2017							
Control	344±1.3	1,69±0,2	26±1	0,96±0,12	40,2±0,6	26,1±0,60	
Aquasorb (20 kg/ha)	352±1.2	1,75±0,2	28±1	1,05±0,10	40,8±0,6	28,2±0,60	8,0
Aquasorb (40 kg/ha)	362±1.1	1,81±0,2	31±2	1,15±0,13	41,4±0,5	31,9±0,50*	22,2
Control + N_{45}	351±1.2	1,78±0,2	27±2	1,01±0,20	40,6±0,6	28,1±0,60	7,7
Aquasorb (20 kg/ha) + N_{45}	366±1.1	1,87±0,2	32±1	1,17±0,14	41,6±0,5	32,7±0,56**	25,3
Aquasorb (40 kg/ha) + N_{45}	372±1.3	1,91±0,1	33±2	1,22±0,20	42,0±0,5	34,8±0,54**	33,3

*, ** Difference between the treatment and control is statistically significant at $p < 0.05$ and $p < 0.01$, respectively.

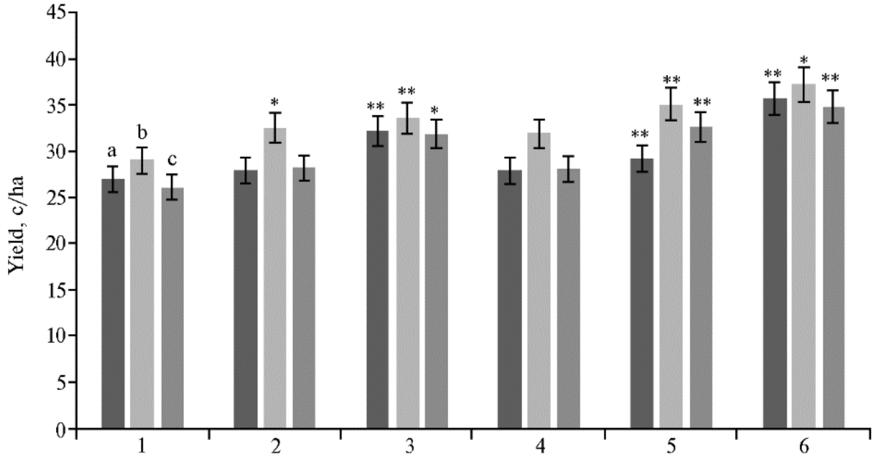

Рис. 2. Yieds of winter wheat (*Triticum aestivum* L.) cv. Steklovidnaya 24 in 2015 (a)**, 2016** (b), and **2017** (в) **as influenced by Aquasorb** (Frnace) **polymer gel introduced into the soil:** 1 — control (withoutabsorbent and nitrogen fertilizers), 2 — Aquasorb (20 kg/ha), 3 — Aquasorb (40 kg/ha), 4 — N_{45}, 5 — N_{45} + Aquasorb (20 kg/ha), 6 — N_{45} + Aquasorb (40 kg/ha) ($n = 4$, M±SEM; field test, zone of insufficient moisture of the Republic of Kazakhstan).
*, ** Difference between the treatment and control is statistically significant at $p < 0.05$ and $p < 0.01$, respectively.

Winter wheat yields in 2017 were lower than in 2015 and 2016 as 2017 was very wet (610.9 mm of rainfall). Yields significantly increased with hydrogel at a dose of 40 kg/ha ($p < 0.01$) and with gel doses of 20 and 40 kg/ha in combination with nitrogen supplementation ($p < 0.05$) (see Fig. 2). The increase in yield ranged from 8.0% to 22.2% without ferilizers, with fertilizers, it was in the range of 16.4-23.8% (Table 3).

In 2015, for the growing season of winter wheat, the sum of average daily temperatures was 2036.4 °C with 305.0 mm precipitation which exceeded the value of the long-term average data (231.6 mm) by 73.4 mm. In this year, which was characterized as moderately dry, nitrogen fertilization with N_{45} had a positive effect on the productive tillering with an increase in the index from 1.94 in control up to 2.02 and 2.10 (under Aquasorb doses of 20 and 40 kg/ha). At a dose of 20 kg/ha, the number of grains per ear increased from 27 to 32 without nitrogen fertilization and from 29 to 35 with nitrogen fertilization, grain weight per ear icreased from 0.98 g to 1.14 g and from 1.03 g to 1.22 g. respectively.

In 2016, due to the warm winter with little snow, active growth and development of plants was observed (average daily air temperature +12.9 °C, maximum +21.3 °C and minimum +4.8 °C). During the growing season, the sum of average daily temperatures amounted to 2126.2 °C, exceeding by 465.1 °C the long-term average values (1694.7 °C). Natural moisture reserves exceeded the 2015 level by 305.9 mm and the long-term average (231.6 mm) by 379.3 mm. Moisture conditions in 2016 were favorable, so winter wheat plants grew and developed actively, which provided them with high productive tillering (from 1.83 to 2.07), high grain number per ear (30 in control vs. 35 when applying hydrogel and fertilizers). At the same time, the weight of grain per ear was high - from 1.04 to 1.27 g. The weight of 1000 grains in the control was 40.9 g; 41.9 g, that is, the increase to the control amounted to 0.5 g and 1.0 g. During the spring nitrogen fertilization at a dose of 45 kg/ha, the formation of a grain mass of 41.5 g in the control and 42.0 and 42.7 g when adding hydrogel.

In 2017, there was slight heat deficit (1981.3 °C), especially in the spring months, due to a significant amount of natural precipitation (385.2 mm). In summer, on the contrary, the increased thermal regime, combined with a lack of precipitation, negatively affected the formation of the yield and grain quality. Due to the underdeveloped short ear, shrunk grains (from 26 to 33 pcs.) were formed. The grain weight per ear was the lowest in the control without fertilizers (0.96 g). The introduction of hydrogel at a dose of 20 kg/ha contributed to an increase in the weight of grain per ear to 1.05 g. An increase in the rate of hydrogel to 40 kg/ha ensured an increase in this indicator to 1.15 g. Nitrogen supplementation increased grain weight per ear from 1.01 g in control to 1.17 and 1.22 g. In general, grain weight per ear was the lowest compared to other years of research. Similarly to the indicator considered above, the 1000-grain weight also was the lowest compared to 2015 and 2016 and in the experiment ranged from 40.2 g in the control to 42.0 g in the test treatments at a hydrogel rate of 40 kg/ha and fertilizers.

Correlation analysis (Table 4) showed a close relationship between yield and yield components in the semi-arid period in all variants of the experiment. When applying 20 kg/ha of Aquasorb hydrogel to the soil, an inverse correlation occurred between the yield and the grain weight per ear (r = –0.99) and 1000-grain weight (r = –0.98), when applying 40 kg/ha. A close inverse relationship was established with the number of grains per ear (r = –0.99) and the grain weight per ear (r = –0.87). Wheat yield also had a close inverse relationship with the number of grains per spike (r = –0.83) in the Aquasorb polymer variants at the application rates of 20 and 40 kg/ha together with nitrogen fertilizers. In humid and moderately humid years, the dependence of yield on yield structure indicators was also strong (r = 0.84-0.99). In these years, the yield had a less close correlation with the number of grains per ear (r = –0.54; r = –0.59). The critical r value at the 5% significance level is 0.71.

4. Correlation coefficients (*r*) between grain yield and yield components in winter wheat (*Triticum aestivum* L.) cv. Steklovidnaya 24 as influenced by Aquasorb (Frnace) **polymer gel introduced into the soil** (field tests, zone of insufficient moisture of the Republic of Kazakhstan, 2015-2017)

Productive stems	Productive tillering	Grains per ear	Grain weigh per ear	1000-grain weight
		Y = f (control)		
0.83*	–0.87*	0.90*	–0.90*	–0.87*
		Y = f (Aquasorb, 20 kg/ha)		
0.99*	0.78*	0.87*	–0.99*	–0.98*
		Y = f (Aquasorb, 40 kg/ha)		
0.86*	0.64	–0.99*	–0.87*	0.99*
		Y = f (N_{45})		
0.84*	–0.92*	0.70	0.99*	0.94*
		Y = f (Aquasorb, 20 kg/ha + N_{45})		
0.69	0.91*	–0.83*	0.99*	0.99*
		Y = f (Aquasorb, 40 kg/ha + N_{45})		
0.77*	0.79*	–0.83*	0.98*	–0.80*

* * Correlation coefficients are statistically significant at p = 0.05 (critical r value at 5% significance level is 0,71).

Therefore, to increase the productivity of winter wheat, the Aquasorb absorbent should be used at a dose of 20 kg/ha in moderately humid and humid years and 40 kg/ha in moderately dry years in combination with early spring fpplication with nitrogen fertilizers.

Thus, our studies carried out under simulated soil drought showed that with hydrogel incorporated into the root layer (10-12 cm), the yield of grain crops differed slightly from the control (an increase of only 3-4%), while when the hydrogel was introduced to a depth of 20-22 cm, it was 25.0-27.7%. When using hydrogels, the productive tillering, the number of grains per ear, and, to the greatest extent, the 1000-grain weight changed significantly, and the weight of grain per ear and the 1000-grain weight had a great influence on the crop yield. In the experiments of N. Kilic et al. [29], it was noted that under arid conditions, the most sensitive indicator of the crop structure to high temperatures and drought is the number of grains per spike. A positive and significant correlation was established between the yield, the number of grains per ear and the weight of 1000 grains. Another study [36] showed that the addition of Sky Gel to the soil at doses of 4, 8 and 12% led to an increase in the number of grains per ear from 45.26 in the control to 48.94, 50.03 and 51.93 pcs. It was found that of 1000-grain weight increased due to an increase in the amount of Sky Gel, the highest value (4.11 g) was obtained when using Sky Gel at a dose of 12%, the lowest (3.43 g) in the control.

The increase in the effectiveness of the hydrogel with a deeper introduction into the soil, noted by us during controlled drought, indirectly indicates the dependence of its ameliorative properties on the water regime of the environment. This is fundamentally consistent with the data that we obtained in field experiments. In the zone of rain-fed agriculture in the southeast of Kazakhstan, when Aqusorb was applied to the root layer (10-12 cm), the effect of the hydrogel and the optimal modes of its application (doses and combination with nitrogen fertilizers) largely depended on the natural supply of moisture to the soil. The gel significantly affected all the studied elements of the structure of the winter wheat crop. J. Grabinski et al. [30] found that the yield of winter wheat largely depends on the weather conditions of the year under study and the dose of hydrogel. The highest yield was obtained when applying an increased dose (30 kg/ha) of the polymer. In addition, the authors note that the 1000-grain weight was significantly higher when treated with doses of 20 kg/ha and 30 kg/ha of hydrogel compared to minimum dose (10 kg/ha) and control. The use of the hydrogel did not affect the number of plants and the number of productive stems of winter wheat, but significantly increased the number of grains per ear, the 1000-grain weight and, consequently, the crop yield. Chinese scientists L. Yan et al. [26] found that the use of Aqusorb polymer in combination with nitrogen fertilizers could mitigate the effects of drought on winter wheat yields.

It should be noted that under controlled drought when the polymer gel was applied to a depth of 20-22 cm, and in field experiments under sufficiently favorable moistening conditions, productive tillering, grain weight per ear, and 1000-grain weight most closely correlated with yield.

So, under the conditions of simulated soil drought, the domestic hydrogels Ritina-10 and V-415 K when placed at a depth of 20-22 cm have a statistically significant effect ($p < 0.001$ and $p < 0.01$, respectively) on the yield value (an increase by 25.0-27.7%) and yield components of grain crops. For Ritin-10, the yield values correlated inversely with the number of productive stems ($r = -0.83$), the number of grains per ear ($r = -0.78$) and the grain weight per ear ($r = -0.78$), for V-415 K hydrogel, with productive tillering ($r = 0.70$), the grain weight per ear ($r = 0.74$) and the 1000-grain weight ($r = 0.71$). The hydrogel introduced into the root-inhabited soil layer (10-12 cm) dried up without irrigation and did not act as a water-retaining soil additive. Under field conditions, the Aquasorb hydrogel (20 kg/ha) + N_{45} ($p < 0.05$) and Aquasorb (40 kg/ha) + N_{45} ($p < 0.01$) increased the yield of wheat. In the semi-arid period, with Aquasorb 20 kg/ha, the yield had a close inverse correlation with the weight of grain per ear ($r = -0.99$) and the 1000-grain weight ($r = -0.98$). For Aquasorb 40 kg/ha, the yield showed a close inverse correlations with the grain number per ear ($r = -0.99$) and the grain weight per ear ($r = -0.87$). Grain yield also closely correlates with the number of grains per ear ($r = -0.83$) when Aquasorb (20 kg/ha and 40 kg/ha) was applied together with nitrogen fertilizers. In wet and moderately wet years, a strong dependence occurred of yield on the crop structure parameters occurred ($r = 0.84$-0.99). Therefore, in the field trials during a dry growing season, it is necessary to apply a high dose of hydrogel (40 kg/ha) in combination with nitrogen fertilizers. In moist and moderately humid growing seasons, a dose of 20 kg/ha is sufficient when using with nitrogen fertilization.

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EFFECT OF BIOCHAR APPLICATION ON VARIABILITY OF THE POLYPHENOLOXIDASE AND PEROXIDASE ACTIVITY OF SOD-PODZOLIC SOIL UNDER LOW AND HIGH FERTILITY

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Abstract

Biochars are considered as an attractive tool in agriculture for carbon sequestration and improvement of soil functions. Biochar addition to soils can raise the pH, increase the organic carbon content, enhance nutrient retention, and increase microbial biomass. The introduction of biochar to different soils is an irreversible action. After entering the soil environment, the so called "aging" of biochar begins, due to the water-physical processes occurring in the soil, e.g., moistening, drying, freezing and thawing. Therefore, it is necessary to understand the directions of various changes occurring in the soil when using this ameliorant. A two-year field experiment to study the effect of biochar on the dynamics of some soil enzymes during of biochar aging was performed with the aim to reveal mechanisms of interaction between soil and biochar and to justify the sensitiveness of enzymes on a biochar amendment to the soil. The studied loamy sand Spodosol soil had medium and high soil quality. The experimental design included the soil (control) and the soil with 20 t/ha biochar introduced in the top arable layer (0-10 cm) of 4 m^2 plots in 3 replicates. The impact of birch (*Betula* spp.) biochar produced by fast pyrolysis at 600 °C was studied. Chemical characteristics of the biochar were as follows: $C_{org.}$ — 88.9 %, $N_{tot.}$ — 0.43 %, H — 3.2%, O — 5.1 %, pH_{H_2O} 8.3, water content — 3.1 %, ash content — 1.8 %. In 2019, a seed mixture of oat (*Avena sativa* L.) cv. Borrus and common vetch (*Vicia sativa* L.) L'govskii cv. was cultivated on the plots at the rate of 200 kg/ha (or 85 g per 4 m^2). In 2020, white lupine (*Lupinus albus* L.) cv. Dega was cultivated as green manure for winter wheat at the rate of 200 kg/ha. Soil and biochar samples were collected from a 0-10 cm layer of the humus horizon in May to August (at 14-day intervals) with an Endelman soil drill (Royal Eijkelkamp B.V., the Netherlands). The activity of peroxidase (EC 1.11.1.7) and polyphenoloxidase (EC 1.10.3.1) was assessed by the photocolorimetric method (λ = 440 nm and λ = 590 nm, respectively), and the assessment of temporal changes in the oxidation of the surface of biochar was studied by IR spectrometry (an FSM 2202 Michelson spectrometer, Infraspek, Russia). The biochar was found to increase the activity of the studied enzymes, on average by 12-13 %, as compared to the activity in soils without biochar. The peroxidase activity was on average 1.5 times higher than that of the polyphenoloxidase and significantly ($p < 0.05$) depended on the degree of soil quality. The ratio of polyphenoloxidase to peroxidase in the soil with medium soil quality was approximately 20 % lower than in the soil with high soil quality, where the conditions (temperature, humidity, amount of organic matter) were optimal for humus synthesis. It was found that all treatments showed the soil humification factor less than 1, which indicates the predominance of the processes of mineralization of humic substances in the soil over their immobilization. The biochar increased the mineralization of organic matter by 11.5 % compared to soils without biochar. Over the two-year experiment, IR spectroscopy revealed a tendency to an increase in the amount of hydroxyl, carbonyl, and carboxylate groups compared to the initial biochar, which is consistent with the data on the increase in the activity of polyphenoloxidase and peroxidase. Our findings confirm that biochar introduced into the loamy sand Spodosol remained stable during two years and did not significantly affect the enzymatic activity of soils.

Keywords: soddy-podzolic sandy loam soil, biochar, peroxidase, EC 1.11.1.7, polyphenol

oxidase, EC 1.10.3.1, IR spectra of biochar

The use of biochar as a high-carbon porous ameliorant produced from various biomass wastes is of great interest due to its potential benefits for sustainable agricultural development and reducing the negative impact of agricultural activities on the environment [1-4]. During oxygen-free pyrolysis, aliphatic carbon chains in the initial biomass are transformed into stable aromatic ones and can be preserved in the soil for hundreds of years [5]. After entering the soil environment, the so-called aging of biochar begins due to the water-physical processes occurring in the soil - moistening, drying, freezing and thawing [6-10]. Even before being introduced into the soil, biochar, being hydrophobic in its chemical structure, begins to oxidize in the air and increase its hydrophilicity [11]. The hydrophilicity of coal largely depends on the pyrolysis temperature and the type of raw material. For example, the available water content of grapevine-derived biochar increased with increasing pyrolysis temperature, with optimal results achieved at 700 °C [12]. Analysis of wood and grass biochars obtained at 400 and 650 °C and kept both in soil and directly in the air for 15 months showed a relative increase in the amount of O-containing functional groups, including substituted aryl-, carboxyl- and carbonyl-C as a result of abiotic and microbial oxidation of biochar, as well as the loss of O-alkyl groups due to leaching [13].

In addition, the biochemical process of decomposition of biochar by microorganisms begins to operate in the soil [14, 15]. It is limited by environmental factors, the availability of organic compounds necessary for the life of microorganisms, and manifests itself in a change in functional groups on the surface of biochar [16, 17]. Many aspects of the influence of biochar on the components of the natural environment, in particular the direction of biochemical changes occurring in the soil, are still poorly understood.

In the present work, we have established for the first time that a two-year stay of biochar in soddy-podzolic sandy loamy soil contributed to the variability of functional groups on its surface, which resulted in an increase in the amount of hydroxyl (−OH), carbonyl (C=O), and carboxylate (COO−) groups compared to the original biochar and in a tendency to increase the mineralization of organic matter, based on the activity of polyphenol oxidase and peroxidase.

The aim of this work is to assess the variability of polyphenol oxidase and peroxidase activity in agro-soddy-podzolic soil with different degrees of cultivation when biochar is introduced into it, as well as to determine the oxidation of the biochar surface after incubation in the soil.

Materials and methods. Field trials were carried out during the growing seasons of 2019 and 2020 on the territory of the experimental station of the Agrophysical Research Institute (settlement Menkovo, Gatchina District, Leningrad Province).

Experimental 4 m^2 plots were placed on parcels of the Agrophysical Station on soil with a medium- (MLC) and high-level (HLC) cultivation, as well as on control plots without the application of mineral fertilizers. The content of physical clay in the medium cultivated soil was 12-15%, in the highly cultivated soil it was 17-19%. The experimental design included two options in 3 repetitions: control (without biochar) and experiment (soil with biochar at a dose of 20 t/ha, which was applied to the top layer of 0-10 cm). The medium-level cultivated soil, was 1.53% Corg, 0.17% Ntot, with 16.4 mg/kg $N\text{-}NO_3$, 5.6 mg/kg $N\text{-}NH_4$, mobile P_2O_5 accounted for 255 mg/kg, K_2O for 112 mg/kg (according to Kirsanov), pH_{KCl} = 5.3. The high-level cultivated soil was 2.92%Corg, 0.28% Ntot with 22.3 mg/kg $N\text{-}NO_3$, 6.7 mg/kg $N\text{-}NH_4$; mobile P_2O_5 accounted for 994 mg/kg, K_2O for 542 mg/kg, pH_{KCl} = 6.4.

We used biochar from birch (*Betula pendula* Roth) grade Premium A (fraction with a particle size of 0.5-2 cm), produced by rapid pyrolysis at a temperature of 600 °C. The biochar was 88.9% C_{org}, 0.43% N_{tot}, 3.2% H, 5.1% O, pH_{H2O} = 8.3, W_{hc} was 3.1%, ash content 1.8%.

In 2019, a mixture of spring vetch (*Vicia sativa* L.) cv. Lgovsky and spring oat (*Avena sativa* L.) cv. Borrus (30% + 70%) was grown on the plots; sowing (200 kg/ha, or 85 g per 4 m^2) was carried out on May 21. In 2020, white lupine (*Lupinus albus* L.) cv. Degas was grown as green manure for winter wheat (seeding rate of 200 kg/ha, sowing on May 15, plowing on August 16), winter wheat was sown on September 3, 2020.

Meteorological data were recorded at a weather station located 200 m from the experimental site.

Soil and biochar samples were taken with an Endelman soil drill (Royal Eijkelkamp B.V., the Netherlands) from the 0-10 cm layer of the humus horizon with an interval of 14 days from May to August inclusive. The activity of peroxidase and polyphenol oxidase was studied by the photocolorimetric method according to A.Sh. Galstyan respectively but at λ = 440 and λ = 590 nm [18]. The humification coefficient (K_{hum}) was calculated from the ratio of the activity of polyphenol oxidase (PPO) to the activity of peroxidase (PO). To assess temporal changes in the oxidation of the biochar surface, the content of oxygen-containing groups was determined by IR spectrometry. An attachment was used to measure the multiple frustrated total internal reflection with a ZnSe crystal on an FSM 2202 Michelson-type spectrometer (Infraspek, Russia) with self-compensation (it does not require dynamic adjustment in the wavenumber range 7800-600 cm^{-1}).

Statistical data processing was carried out in Microsoft Excel and Statistics 8.0 programs. Mean values (*M*), standard deviations (±SEM) and linear correlation coefficients were calculated at a significance level of $p \leq 0.05$. The significance of differences in mean values was assessed using analysis of variance (ANOVA).

Results. The experiments were carried out in the Non-Chernozem zone of Russia, which belongs to the temperate cold zone [19]. Comparing the growing seasons of 2019 and 2020, we can note the common features of hydrometeorological indicators by months. May was cold, with an average rainfall of up to 80 mm, June was hot and dry, July was warm and humid, August was warm but also humid in 2020 compared to the moderately humid 2019, September was cold and moderately wet. During the growing season of 2019, 451 mm of precipitation fell, in 2020 - 517 mm of precipitation.

The intensity of enzymatic processes depends on the specific conditions prevailing in the soil and is variable. Horizontal variability depends on temperature, humidity, and the intensity of fresh organic input, while the vertical gradient depends on the quality of organic matter and the physical properties of the soil [20]. Polyphenol oxidase (PPO; o-diphenol:oxygen oxidoreductase, EC 1.10.3.1) is one of the most important enzymes involved in the oxidation of aromatic organic compounds and the formation of humus [21].

In our experiments, there was a significant difference ($p < 0.05$) between the variants with different soil cultivation in terms of PPO activity, while the introduction of biochar only led to an insignificant increase in the indicator by 10-13% (Fig. 1). Despite the level of soil cultivation, polyphenol oxidase activity showed an increase in the initial periods of crop vegetation which, as a rule, is due to the active root growth, low soil temperatures, and optimal moisture. By the second decade of June when the soil temperature began to rise but drought was recorded, PPO decreased. By the end of July, with an increase in the amount of precipitation, a significant ($p < 0.05$) increase in PPO activity was noted, and by the end of the growing season, a decrease. Relatively increased values of polyphenol oxidase activity by the end of July are presented as a total result of plant maturation, an increase in the number of microorganisms, and an intensification of decomposition of plant residues under more favorable hydrothermal conditions [22].

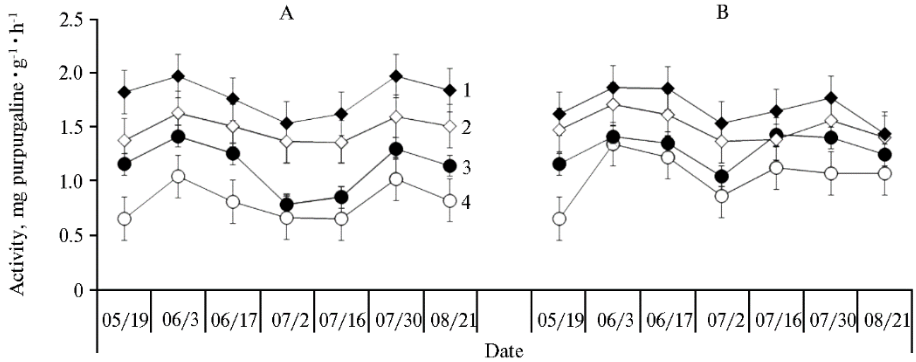


Fig. 1. Activity of polyphenol oxidase (EC 1.10.3.1) **in soddy-podzolic soils with medium- (MLC) and high-level (HLC) cultivation as influenced by biochar from pyrolyzed premium grade birch wood:** A — vetch-oat mixture (2019), B — white lupine (2020); 1 — HLC with biochar, 2 — HLC without biochar, 3 — MLC with biochar, 4 — MLC without biochar (settlement Menkovo, Gatchina District, Leningrad Province).

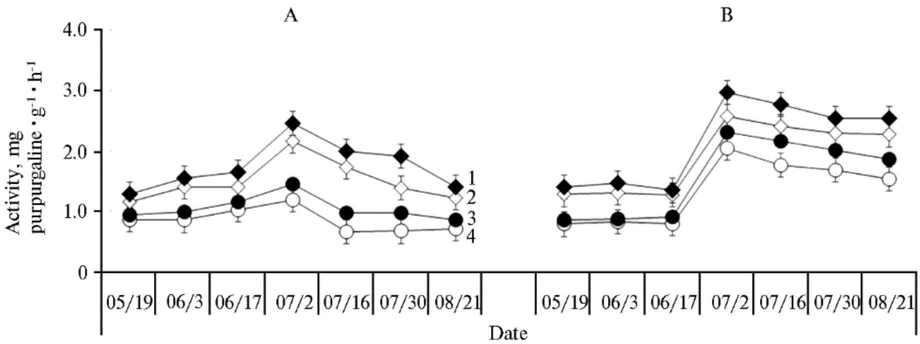
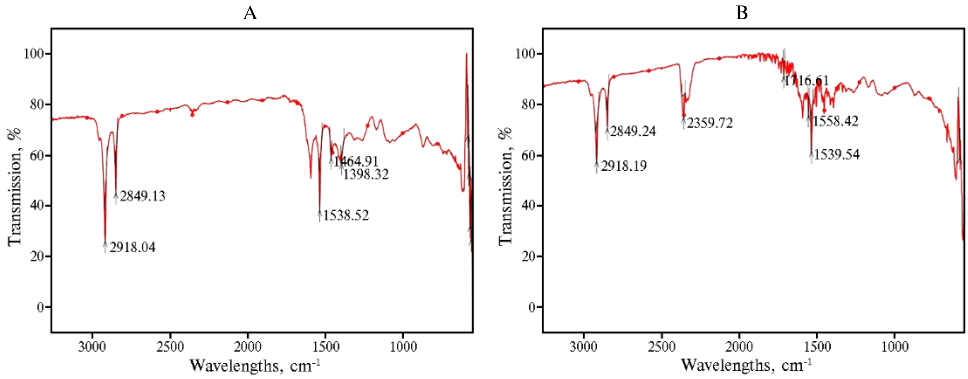


Fig. 2. Activity of peroxidase (EC 1.11.1.7) **in soddy-podzolic soils with medium- (MLC) and high-level (HLC) cultivation as influenced by biochar from pyrolyzed premium grade birch wood:** A —vetch-oat mixture (2019), B — white lupine (2020); 1 — HLC with biochar, 2 — HLC without biochar, 3 — MLC with biochar, 4 — MLC without biochar (settlement Menkovo, Gatchina District, Leningrad Province).

Peroxidase (PO, EC 1.11.1.7) is an enzyme that oxidizes organic matter with the participation of hydrogen peroxide (soil organic matter, including humic substances) [22, 23]. The activity of PO in soddy-podzolic sandy loamy soil was on average 1.5 times higher during the growing season than the activity of PPO, and significantly ($p < 0.05$) depended on the degree of cultivation, that is, the higher the cultivation of the soil, the higher it turned out to be. peroxidase activity (Fig. 2). In addition, in contrast to PPO, the activity of PO increased significantly with increasing temperature.

The introduction of biochar, in addition to influencing the rate of oxygen consumption, had an impact on the production of peroxidase, which increased by 13% both in the soil with MLC and with HLC. The maximum activity of this enzyme in all variants of the experiment was observed in early July, reaching values of 2.3 and 3.0 mg of purpurgalin $\cdot g^{-1} \cdot h^{-1}$ for soil under MLC and HLC, respectively.

The intensity of humus formation is characterized by the humification coefficient (K_{hum}), which is the ratio of polyphenol oxidase activity to peroxidase activity [24]. Calculations showed that in all variants of the experiment $K_{hum} < 1$, which indicated the predominance of the processes of mineralization of humic substances, plant residues, and nonspecific organic compounds in the soil over their synthesis. Kgum. in soil with HLC was about 20% lower than in soil with HLC. The introduction of biochar contributed to the increase in K_{hum}. by an average of 11-12% compared to soils without biochar.



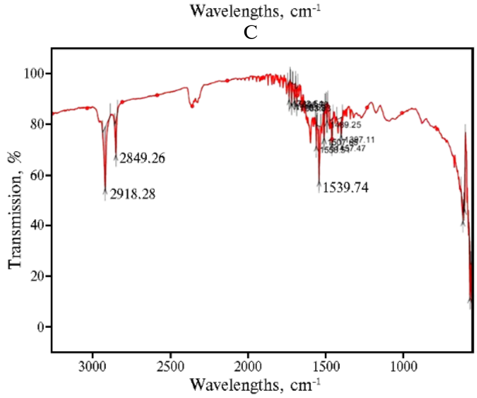


Fig. 3. IR spectra of biochar from pyrolyzed Premium grade birch wood: A — native biochar (control) (May 2019), B — biochar after incubation in soil with a medium-level cultivation (at the end of the growing season, August 2020), C — biochar after incubation in soil with a high-level cultivation (at the end of the growing season, August 2020) (settlement Menkovo, Gatchinsky District, Leningrad Province).

The main changes in the enzymatic activity of soils with biochar can be associated with the transformation of organic functional groups on the surface of biochar (an increase in the number of −COO− and −O− groups), as well as with the ability of biochar particles to absorb H^+ ions. The change in the enzymatic activity of the soil occurs due to the sorption of enzymes on the surface of the biochar with the formation of matrix-enzyme complexes, which are more stable and stable in the soil, have a greater enzymatic activity. Biochar also sorbs various low molecular weight and soluble high molecular weight organic compounds (carbohydrates, organic acids, aromatic compounds, including phenolcarboxylic acids, phenols, etc.) from the soil solution, which can be identified by IR Fourier spectrometry of functional groups. They are localized on the surface of coal and serve as substrates for the activity of microorganisms adsorbed on biochar.

Studies of temporal changes in the oxidation of the surface of biochar made it possible to identify a number of zones in the IR spectra in the absorption mode with an increased intensity of oscillations, which characterize the change in the surface of the coal after it has been in soils. The original coal (Fig. 3, A) had bands in the region of 1500-1100 cm^{-1}, characteristic of the C−O and C=O groups, and bands in the region of 2900 cm^{-1}, which indicated the presence of aliphatic groups, including characteristic of aldehydes (C−H bonds in the state of sp3 hybridization) [25]. A pronounced intensity of the vibrational spectrum was also observed in the region of 1600 cm^{-1}, which is characteristic of the quinoid groups of aromatic compounds.

After incubation of biochar in the soil for 17 months, an increase in the content of various oxygen-containing groups was observed, except for quinoid ones, where the intensity of fluctuations at 1600 cm^{-1} decreased by more than 2 times compared to the control, and the content of aliphatic groups also decreased (bands at 2900 cm^{-1}). The content of hydroxyl (−OH), carbonyl (C=O) and carboxylate (COO−) groups on the biochar surface showed a trend of their increase (1539-1540 cm^{-1}). On the whole, biochar practically remained in a stable state over the two growing seasons, and a significant decrease in the proportion of aliphatic groups with an insignificant dynamics of oxygen-containing groups indicated interaction with the components of the soil solution.

In most studies on the effect of biochar on microbial activity in soils, it has been shown that it increases, but the intensity of the effect depends on the properties of the initial biomass from which biochar is produced, pyrolysis conditions (duration and temperature), and the soil into which biochar is directly applied [1 , 26, 27]. Biomass usually determines the chemical composition, number of macropores, and nutrient content in biochar, while pyrolysis conditions determine changes in the morphology and structure of the surface in the feedstock and the C:H ratio [28].

It should be noted that information on the effect of various biochars on the enzymatic activity and the chemical properties of soils related to it is still contradictory. Since enzymes are proteins, all factors affecting the protein will also affect the activity of the enzyme, and biochar introduced into the soil, as has already been proven, changes gas exchange and the specific surface of the soil, its water-holding capacity and other physical and chemical properties [1, 29]. The large specific surface of biochar helps to adsorb labile substances from the environment, which affects the activity of various enzymes that break down such substances into simple molecules, which are further used by bacteria and fungi in primary and secondary metabolism [30].

In our experiments, we have revealed a general tendency for an increase in the activity of peroxidase and polyphenol oxidase when biochar is introduced into the soil: by an average of 13% (average degree of cultivation) and 12% (high degree of cultivation). The activity of PO was on average 1.5 times higher than the activity of PPO, and significantly ($p < 0.05$) depended on the level of soil cultivation. J. Park et al. [31] and S. Kumar et al. [32] noted the positive effect of biochar on enzymes, as judged by the increased uptake of carbon, nitrogen and phosphorus from the soil. J. Paz-Ferreiro et al. [33], F. Wu et al. [34] and J. Lehman et al. [1] pointed to the negative effects of biochar reducing enzymatic activity compared to soils without biochar. The mechanisms explaining such conflicting observations have not been identified for a long time. It turned out that the sorption of a substance and extracellular enzymes that differ in functional groups on the surface of biochar can enhance or limit the enzymatic reaction, and the characteristics of biochar as a sorbent can change over time [11]. Our work describes the processes that lead to a change in the state of functional groups on the biochar surface over time. Other studies have also shown that the aging of the ameliorant leads to the appearance of more carboxyl functional groups on its surface, a higher oxygen content in biochar and a lower total carbon [35]. F.V.D.N. Tozzi et al. [36] assessed the stability of carbon in biochar and its effect on surfactants when wood biochar was used to sequester carbon in soil. After 120 days of incubation, an increase in the degree of carbon mineralization by 0.4-9.3% (depending on the type of soil) was found, which indicated its high stability. This is also confirmed by our data on a slight change in the carbon content of woody biochar over a two-year stay in the soil.

Oxidation of biochar in soils can lead to an increase in the density of oxygen-containing functional groups on its surface. The high density of oxygen-containing functional groups detected by IR spectroscopy [37] increases the affinity of the biochar surface for the adsorption of water molecules, nutrients, and organic compounds [38-40].

Therefore, for 17 months of the experiment, the activity of polyphenol oxidase (PPO) and peroxidase (PO) in soddy-podzolic sandy loamy soils with the introduction of biochar increased on average by 13% (average degree of cultivation) and 12% (high degree of cultivation) compared with the variants without biochar. The PO activity was on average 1.5 times higher than the PPO activity and significantly ($p < 0.05$) depended on the degree of soil cultivation. The coefficient of humification in the soil with MLC was approximately 20% lower than in the soil with HLC, and in all test variants it turned out to be less than 1, which indicates the predominance of the processes of mineralization of humic substances in the soil over their immobilization. With the introduction of biochar, an increase was observed in the K_{hum} index by 11-12% compared to soils without biochar. In biochar after incubation in the soil, there was a trend towards an increase in the number of hydroxyl (−OH), carbonyl (C=O), and carboxylate (COO−) groups compared to the initial biochar. Based on the results of changes in the values of PPO, PO, and IR spectra, it can be argued that the introduced biochar remained in a stable form and did not significantly affect the enzymatic activity of soils.

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BIOLOGICAL FEATURES OF THE RESPONSE OF FODDER GRASSES TO THE USE OF IODINE ON AGROSOD-PODZOLIC SOILS OF VARIOUS CULTIVATION LEVELS

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Abstract

A geochemical anomaly of iodine deficiency the North-Western region of the Russian Federation negatively affects the yield and quality of marketable products of regional agriculture and feed, the viability and productivity of farm animals, and the health of the population. In this study, for the first time in the conditions of the region, the optimal concentration levels of the KI solution for foliar fertilization and the time period of treatment on the annual and perennial grasses dominating in the structure of the acreage of the Non-Chernozem region were established. Our goal was to study the biological characteristics and evaluate the parameters of responsiveness of forage grasses to changes in the concentration of the KI solution and the period of time of iodine foliar treatments. The research was carried out in 2019-2021 in the Menkovo branch of the Agrophysical Institute (Gatchinsky District, the Leningrad Province). Two micro-field experiments were laid down in the system of a long-term fundamental field agrophysical experiment in the field crop rotation link: potatoes—annual grasses + perennial grasses—perennial grasses of the 1st year of use—perennial grasses of the 2nd year of use. The object of the study was mixed crops. Annual grasses were presented by the oat (*Avena sativa* L.) variety Skakun and the garden vetch (*Vicia sativa* L.) variety Vera, perennial grasses were presented by the red clover (*Trifolium pratense* L.) variety Orpheus and the timothy (*Phleum pratense* L.) variety Leningradskaya 204. Both experiments had a two-factor scheme. Factor A is the degree of cultivation of sandy loam agrosod-podzolic soil (medium-cultivated, well-cultivated and highly cultivated). The scheme of the first experiment on factor B included nine variants of the concentration of the KI solution: 0, 0.005, 0.01, 0.02, 0.04, 0.08, 0.16, 0.32, and 0.64 %. Foliar treatments of annual grasses were carried out in the booting stage of oat, perennial grasses were in the tillering stage. In the second experiment, four variants of the time period of foliar treatment with 0.02 % KI solution were studied by factor B: KI-0 — control without treatment; KI-1 — early treatment in the tillering stage of oats, red clover and timothy; KI-2 — late treatment in the booting stage of oat and in the stage of branching of red clover; KI-3 — two-fold treatments in terms corresponding to variants KI-1 and KI-2. The yield of the aboveground biomass of grasses used for the preparation of feed was counted by a continuous weight method from a 1 m^2 plot. The placement of plots by repetitions and variants was systematic. The repetition in the first experiment was threefold, in the second — sixfold. A chemical-analytical analysis of selected soil and plant samples was carried out. As a result of short-term field experiments, it was found that the responsiveness of forage grasses to the iodine foliar treatment under a geochemical anomaly of iodine deficiency is determined by a combination of weather-climatic and agrochemical soil conditions with biological characteristics of crops and depends on the period of time of treatment and the concentration of the KI solution. For annual grasses, the treatment was more effective in the booting stage of oat (yield increased by an average of 2.49 t/ha, or 29 %; $p \le 0.05$), whereas for perennial grasses in the tillering stage of red clover and timothy (an increase of 3.39 t/ha, or 18 %; $p \le 0.05$). The optimal C_{KI} for the treatment of annual grasses was 0.16 %, regardless of the degree of cultivation of the soil, and of perennial grasses on soils of medium, good and high cultivation was 0.04, 0.08 and 0.16 %, respectively. The increase ($p \le 0.05$) in productivity reached 3.69-9.38 t/ha, or 67-80 %, for annual grasses and 3.91-8.03 t/ha, or 22-30%, for perennial grasses. The positive effect of iodine increased with the optimization of soil and agrochemical conditions to good and high cultivation by 68 and 128 %. Due to high tolerance to the concentration of the KI solution, toxic effect was detected only at C_{KI} 0.32-0.64 %, when crop losses reached 19 %. Legume types of herbs were more sensitive to the excess of iodine. The reduction of iodine toxicity in the experiments was facilitated by an increase in soil cultivation and a change in the botanical composition of crops with an increase in the proportion of cereals. Perennial grasses accumulated 9 % less iodine than annual ones. In the variants with optimal C_{KI}, the iodine content in the aboveground biomass of annual and perennial grasses increased on average from 119 and 88 to 766 and 628 μg/kg, that is, 6.4-fold and 7.1-fold. The accumulation of nitrates, on the contrary, decreased ($p \leq 0.05$) by 13 % in annual and 11 % in perennial grasses. The maximum level of iodine accumulation in the green mass of annual grasses were about 600 on medium cultivated soil, 900 on well-cultivated soil, and 1500 μg/kg on highly cultivated soil. In perennial grasses less sensitive to soil cultivation, this value practically did not depend on soil and agrochemical conditions and amounted to 900 μg/kg. One of the signs of iodine toxicity was a 23-33 % ($p \leq 0.05$) increase in the content of nitrates in products.

Keywords: fodder grasses, annual grasses, perennial grasses, iodine, nitrates, iodine fertilizers, agrosod-podzolic soil, cultivation, productivity

Improving the quality of agricultural products is one of the main challenge [1, 2]. The global problem is still the lack of iodine, due to the geochemical features of its distribution and behavior in the environment. The implementation of long-term state programs for the prevention of iodine deficiency made it possible to get rid of the most severe human pathologies caused by chronic iodine deficiency in food, but did not fully solve the problem either in Russia [3, 4] or in the European Union [4, 5]. Spatial heterogeneity of soils for iodine, local soil contamination with a radioactive isotope in Belarus and Russia [6-8], and limited availability of iodine-fortified foods for a part of the population, mainly rural, along with the low content of the element in soils and waters, complicate iodine deficiency [9].

Animal husbandry, which is the main commercial sector of agriculture in the Non-Chernozem region [2], also faces the negative consequences of iodine deficiency in feed [10, 11]. Despite the fact that the world has accumulated extensive scientific data on the effective use of iodine microfertilizers [12-15], including together with selenium [15-19], in the North-West region of Russia, various aspects of their use have been systematically studied only in the Kaliningrad region [20]. It was shown that even the coastal position of the region does not allow compensating for the deficiency of iodine in arable soils caused by soil genesis.

Multi-scale field experiments revealed the advantage of foliar treatment with a iodine microfertilizer over its application to the soil [21-23], as well as increased toxicity and some superiority of the iodide (I^-) over the iodate (IO^{3-}) [24-27]. It has been found out that iodine in optimal concentrations, interacting with amino acids, proteins and enzymes, stimulates the synthesis of sugars and proteins, enzymatic (peroxidase and oxidoreductase) and antioxidant activity of plant cells [28, 29]. By enhancing the biosynthesis of tryptophan and its transamination into indole auxins which promote meristem cell elongation, iodine also promotes nutrient transport in plants [30].

Thereof, in optimal concentrations, iodine activate the production processes, increase tolerance of agrocenoses to negative biotic and abiotic factors, and improve the quality of commercial products in terms of accumulation of iodine, proteins, vitamins, and sometimes sugars [18, 19, 28]. On the contrary, an excess of iodine inhibits part of the nitrogen cycle enzymes (nitrate reductase, glutamate dehydrogenase) and protein biosynthesis, including through increased productucion of the phytohormone ethylene, and promotes the accumulation of nitrates [31, 32]. The optimal dosage, accumulation in plant biomass, and the toxicity of iodine depend on the biological properties of crops, varieties, as well as soil and agrochemical conditions [26, 27, 33, 34].

Despite the quite obvious theoretical prerequisites, the problem of improving the iodine status of fodder crops has been ignored by the regional scientific community for many years. In fact, there are no recommendations on the types, dosages, terms and modes of treatment of forage grasses with iodine microfertilizers. Note, biofortification with iodine increases the yield of grasses and improves their nutritional value, which, in turn, increases the productivity of livestock and the quality of dairy products [11, 30], as iodine, covalently binding to milk casein, forms a complex which is physiologically most suitable and valuable for humans.

This paper is the first to indicate the concentrations of KI solutions for foliar application and timing of threaamaents optimal under the conditions of the Leningrad Province for the annual and perennial grasses that dominate in the sown areas of the Non-Chernozem Region.

Our goal was to evaluate the biological response of forage grasses to various KI concentrations and timing of foliar treatment with potassium iodide.

Materials and methods. The studies were carried out in 2018-2021 in the Menkovsky branch of the Agrophysical Research Institute (API, Gatchinsky District, Leningrad Province). Two microfield tests were incorporated in a long-term fundamental experiment (agrophysical station) on the field crop rotation, the potatoes—annual grasses + perennial grasses—perennial grasses of the 1st year of use—perennial grasses of the 2nd year of use.

Of grasses for mixed crops, annual herbs (oats *Avena sativ*a L. cv. Skakun of the FRC Nemchinovka, Russia; vetch *Vicia sativa* L. cv. Vera of Federal Williams Research Center of Forage Production & Agroecology, Russia) and perennial herbs (red clover *Trifolium pratens*e L. cv. Orfey of Rudnitsky FARC of the North-East, Russia; meadow timothy grass *Phleum pratense* L. cv. Leningradskaya 204 of Belogorka Leningrad Research Institute of Agriculture — a branch of the Lorkh Federal Research Center for Potatoes, Russia) were used.

Both experiments had a two-factor scheme. Factor A was the degree of cultivation of sandy loamy agro-podzolic soil (medium-cultivated, well-cultivated and highly cultivated) due to the long-term use of organic fertilizers and lime. The humus content in the topsoil is 2.51, 3.48 and 4.46%, respectively;mobile phosphorus compounds amounted to 199, 325 and 364 mg/kg, mobile potassium compounds to 49, 162 and 274 mg/kg, total iodine to 0.94, 1.22 and 1.48 mg/kg, with pH_{KCl} 5.12, 5.99 and 6.25.

The first experiment included nine concentrations of the KI working solution (C_{KI}) as factor B (0, 0.005, 0.01, 0.02, 0.04, 0.08, 0.16, 0.32, and 0.64%). Foliar treatment of annual grasses was carried out at the time of oats stem extention, of perennial grasses at tillering. In the second experiment, the timing of foliar treatment uding 0.02% KI solution was as follows: KI-0 for control without treatment, KI-1 for early treatment at tillering of oats, red clover and timothy grass, KI-2 for late treatment at stem extention of oats and branching of red clover, KI-3 for double treatment, the timing is as for KI-1 and KI-2.

Spraying was carried out in the evening in calm weather using a backpack sprayer STIHL SG51 (Andreas Stihl AG & Co. KG, Germany) with a working fluid flow rate of 30 ml/m^2. The working solution was prepared using crystalline KI, chemically pure (Troitsky Iodine Plant, Russia).

The yield of aboveground grass biomass used for fodder preparation was taken into account by a continuous weight method from a plot of 1 m^2. Placement of plots according to repetitions and variants is systematic. The repetition in the first experiment was 3-fold, in the second 6-fold.

Chemical assays of 1-1.2 kg bulk green mass samples of harvested grasses composed of 10 individual samples, was carried out in 3 replications using standardized methods (the study was carried out by accredited laboratories of GSAS

Pskovskaya and API. The content of iodine in the dry green mass of annual and perennial grasses was determined according to GOST 31660-2012 (Moscow, 2012) by the stripping voltammetric method after dry ashing and dissolution of the precipitate in sulfuric acid using Ecotest-VA-iodine (OOO Ekoniks-Expert, Russia). The concentration of nitrates was estimated according to GOST 13496.19-2015 (Moscow, 2016) by the ionometric method after extraction with a 1% solution of potassium alum (an HI98191, Hanna Instruments, Germany).

Statistical processing was carried out by the dispersion method after checking the compliance of the sample with the normal distribution law in the Statistica 7.0 software package (StatSoft, Inc., USA). The significance of differences in deviations was assessed at a 5% significance level using Fisher's *F*-test expressed via LSD_{05} for each factor and their interaction. The tables and figures show the average values (*M*) and a confidence interval with a standard error of the mean (±SEM).

Results. A significant impact on the production process of unfavorable weather and climatic conditions at the beginning of the growing season was shown in all years of research. The early summer drought characteristic of the region [2] reduced the hydrothermal coefficient in June, during which both crops have the most intensive growth period, to 0.2-0.7 units. Annual grasses and perennial grasses of the 1st year of use were especially hard hit. From the critical consequences of the June drought in 2021, the sowing of perennial grasses of the 2nd year of use was saved by waterlogging of the soil in April-May.

A direct consequence of this was not only the unusually low productivity of annual grasses, but also their very high responsiveness to increasing the effective fertility of agro-soddy-podzolic soil (Table 1). Due to the optimization of the water and potassium regimes of the soil, which control the watering of the cell cytoplasm, as the cultivation of the soil increased to good and high, the productivity of annual grasses increased by 59 and 195%, respectively ($p \leq 0.05$). For perennial grasses, similar parameters for 2 years averaged 52 and 72% ($p \leq 0.05$).

Under such a critical weather and climatic parameters, the responsiveness of annual grasses to foliar feeding with a solution of potassium iodide turned out to be unexpectedly high, 27% vs. 13% ($p \leq 0.05$) for perennial grasses. The optimal C_{KI} for annual grasses was 0.16%, regardless of the degree of soil cultivation, and for perennial grasses on soils of medium, good, and high cultivation, it was 0.04, 0.08 and 0.16%, respectively. The optimal concentration of the working solution of potassium iodide in this experiment turned out to be significantly higher than previously on potato crops [34], oats [36] and winter rapeseed [37]. The increase in productivity from foliar treatment with iodine reached 3.69-9.38 t/ha, or 67-80% ($p \leq 0.05$), for annual grasses and 3.91-8.03 t/ha, or 22-30% ($p \leq 0.05$), for perennial grasses. The probable reason for such a significant effect of this technique, along with compensation for the lack of iodine in the soil, was the presence of potassium in the composition of the microfertilizer, which plays one of the key roles in plant drought resistance. The protective physiological function of iodine itself is largely associated with an increase in the antioxidant activity of the cell cytoplasm by stimulating the synthesis of glutathione, ascorbic acid, and phenolic compounds and preventing the oxidative degradation of proteins, nucleic acids, and carbohydrates [25, 38, 39]. A.V. Sindireva et al. [36] in pot trials proved a threefold increase in catalase activity in the aboveground biomass of oats.

The positive effect of iodine on both crops increased markedly as the soil and agrochemical conditions were optimized. The increase in the yield of grass green mass from foliar feeding with iodine at the optimal concentration during the transition from medium to good and high degree of cultivation increased by 33-102 and 105-154%, respectively ($p \leq 0.05$).

1. Yields of annual and perennial grasses depending on cultivation level of agro-podzolic soil and the KI concentration (C_{KI}) (n = 3, M±SEM, Menkovsky branch of the Agrophysical Research Institute, Gatchinsky District, Leningrad Province, 2019-2020)

Variant		Annual grasses			Perennial grasses		
soil cultivation (factor A)	C_{KI}, % (factor B)	yield, t/ha	Δ due to KI		yield, t/ha	Δ due to KI	
			t/ha	%		t/ha	%
Medium cultivated	0	4.62±0.32			17.50±0.79		
	0.005	5.10±0.27	0.49	11	17.79±0.51	0,29	2
	0.01	5.19±0.28	0.58	12	18.64±0.77	1,14	7
	0.02	5.98±0.19	1.36	30	20.39±0.84	2,88	16
	0.04	6.49±0.23	1.87	41	21.42±0.46	3,91	22
	0.08	6.61±0.09	1.99	43	22.28±0.64	4,78	27
	0.16	8.30±0.22	3.69	80	22.38±0.39	4,88	28
	0.32	7.45±0.17	2.84	61	20.07±0.58	2,57	15
	0.64	5.76±0.17	1.15	25	16.94±0.51	–0,57	–3
Well cultivated	0	7.36±0.10			26.54±1.10		
	0.005	7.59±0.07	0.23	3	26.86±0.97	0,32	1
	0.01	8.38±0.26	1.02	14	27.79±0.57	1,25	5
	0.02	9.44±0.29	2.08	28	29.51±0.32	2,97	11
	0.04	10.65±0.25	3.29	32	32.65±0.39	6,11	23
	0.08	11.45±0.27	4.09	56	34.43±0.34	7,89	30
	0.16	12.26±0.34	4.90	67	34.80±0.56	8,26	31
	0.32	11.61±0.29	4.25	69	30.74±0.34	4,20	16
	0.64	10.41±0.39	3.05	37	28.26±0.68	1,72	6
Highly cultivated	0	13.65±0.31			30.03±0.71		
	0.005	14.12±0.32	0.47	3	30.29±0.68	0,25	1
	0.01	15.38±0.27	1.73	13	31.38±0.45	1,35	4
	0.02	17.07±0.34	3.42	25	33.40±0.71	3,37	11
	0.04	19.25±0.34	5.60	41	33.77±0.33	3,73	12
	0.08	20.94±0.44	7.31	54	36.62±0.41	6,59	22
	0.16	23.03±0.55	9.38	69	38.06±0.68	8,03	27
	0.32	22.12±0.56	8.47	62	34.55±0.90	4,52	15
	0.64	18.95±0.29	5.30	39	30.72±0.93	0,69	2
LSD_{05}							
factor A		0.31			1.39		
factor Б		0.53			1.48		
A×Б		0.92			$F_{факт.} < F_{05}$		

N o t e. Annual grasses — oat (*Avena sativa* L.) cv. Skakun and common vetch (*Vicia sativa* L.) cv. Vera, perennial grasses — red clover (*Trifolium pratense* L.) cv. Orfey and meadow timothy grass (*Phleum pratense* L.) cv. Leningradskaya 204.

In contrast to potatoes which showed acute sensitivity to excess iodine already at C_{KI} 0.06-0.08% [34], the response of herbs was more plastic. Legume species (common vetch and red clover) responded more sharply to the increase in C_{KI}, i.e., at the 0.64% concentration, marginal leaf necrosis was detected, similar to that described by P.G. Lawson et al. [22]. Yield losses from excess iodine vs. optimal concentrations averaged 19% ($p \leq 0.05$) and were comparable to a 20% decrease in lettuce and kohlrabi yields reported by P.G. Lawson et al. [22].

The decrease in the sensitivity of crops to an excess of KI was facilitated by the improved soil cultivation which was associated with botanical composition of crops. If on medium cultivated soil the common vetch in crop biomass reached 71-78%, on highly cultivated soil 43-47%, for red clover, the distribution was more even, 76-88 and 69-73%, respectively. Therefore, annual grasses tolerated excess iodine better on cultivated soils than perennial herbs. In addition, according to C.L. Mackowiak et al. [40), due to the enrichment with humic acids, cultivated soils have a significantly higher potential for iodine detoxification than less humus soil types.

Due to the biological specificity of development, crops responed differently to the timing of foliar feeding with 0.02% KI solution (Table 2).

For annual grasses, the late spraying at booting stage was more favorable (an increase in yield averaged 2.49 t/ha, or 29%), for perennial grasses, it was earlier foliar reatmenr at tillering (with an increase by 3.39 t/ha, or by 18%)

($p \leq 0.05$). An obvious reason for this was the poor development of oat and vetch plants in the tillering phase (the projective soil cover is less than 5%), as a result of which only an insignificant part of the fertilizer got to the plants. In the same phase, in red clover and timothy grass, the projective soil leaf cover exceeded 60%, which, in combination with a favorable water regime, ensured the advantage of this variant.

2. Yields of annual and perennial grasses depending on cultivation level of agro-podzolic soil and the timing of KI application (n = 6, M±SEM, Menkovsky branch of the Agrophysical Research Institute, Gatchinsky District, Leningrad Province, 2019-2020)

Variant		Yield, t/ha	ΔYiled					
soil cultivation (factor A)	timing of treatment (factor B)		total		due to soil cultivation		due to KI	
			t/ha	%	t/ha	%	t/ha	%
Annual grasses								
Medium cultivated	KI-0	4.53±0.18						
	KI-1	5.94±0.08	1,41	31			1,41	31
	KI-2	6.27±0.12	1,74	38			1,74	38
	KI-3	5.29±0.14	0,76	17			0,76	17
Well cultivated	KI-0	7.44±0.12	2,91	64	2,91	64		
	KI-1	9.39±0.16	4,86	107	3,45	58	1,95	26
	KI-2	9.65±0.20	5,12	113	3,38	54	2,21	30
	KI-3	8.64±0.18	4,11	91	3,35	63	1,20	16
Highly cultivated	KI-0	13.88±0.39	9,35	206	9,35	206		
	KI-1	14.82±0.11	10,29	227	8,88	149	0,94	7
	KI-2	17.37±0.19	12,84	283	11,10	177	3,49	25
	KI-3	12.96±0.15	8,43	186	7,67	145	–0,92	–7
LSD_{05}			2,95		0,92		1,97	
Perennial grasses								
Medium cultivated	KI-0	15.47±0.35						
	KI-1	19.97±0.59	4,50	29			4,50	29
	KI-2	18.90±0.50	3,43	22			3,43	22
	KI-3	20.50±0.19	5,03	33			5,03	33
Well cultivated	KI-0	19.69±0.37	4,22	27	4,22	27		
	KI-1	22.78±0.26	7,31	47	2,81	14	3,09	16
	KI-2	21.48±0.46	6,01	39	2,58	14	1,79	9
	KI-3	23.28±0.33	7,81	50	2,78	14	3,59	18
Highly cultivated	KI-0	20.52±0.44	5,05	33	5,05	33		
	KI-1	23.10±0.16	7,63	49	3,13	16	2,58	13
	KI-2	21.67±0.65	6,20	40	2,77	15	1,15	6
	KI-3	23.14±0.39	7,67	50	2,64	13	2,62	13
LSD_{05}			1,03		0,52		0,60	

Note. Annual grasses — oat (*Avena sativa* L.) cv. Skakun and common vetch (*Vicia sativa* L.) cv. Vera, perennial grasses — red clover (*Trifolium pratense* L.) cv. Orfei and meadow timothy grass (*Phleum pratense* L.) cv. Leningradskaya 204.

The response to repeated spraying also differed drastically. While perennial grasses showed a positive trend towards higher yields after treatment, annuals showed very noticeable toxicity. It is not yet possible to find an unambiguous explanation for the latter, given the high efficiency of iodine in the first experiment.

Possessing a high physiological activity, iodine significantly influenced the quality of the green mass of grasses used for fodder production. Of the nine parameters studied (dry matter, nitrogen, phosphorus, potassium, crude protein, crude fiber, crude ash, iodine and nitrates), two turned out to be the most sensitive, the content of iodine and nitrates (Fig. 1). As in the work of V.I. Panasina et al. [37], in winter rapeseed, iodine in most of the studied dosfges was absorbed by the surface of grass leaves due to a barrier-free mechanism. In the first experiment, an almost linear functional dependence of the iodine content in the green mass was established up to C_{KI} 0.16% for perennial grasse, up to C_{KI} 0.04% for annual grasses on medium cultivated soil and up to C_{KI} 0.32% for annual grasses on well and highly cultivated sois. As a result, the maximum parameters of iodine accumulation in the green mass of annual grasses amounted to approx. 600 μg/kg on medium cultivated soil, 900 μg/kg on well-cultivated soil, and 1200 μg/kg on highly cultivated soil. For perennial grasses less sensitive to soil cultivation, this value was practically independent of soil and agrochemical conditions and amounted to 900 μg/kg. These parameters can be taken as the limit at which irreversible toxic reactions are found in herbs [22]. These values significantly exceeded those previously reported by A.V. Sindireva et al. [36] ofor oat, which is probably due to the specific conditions of the pot trials. However, R. Li et al. [12] succeeded in biofortifying horticulture products to an iodine content of 1330-4000 μg/kg, and an absolute maximum of 10000 μg/kg was achieved on tomatos [41].

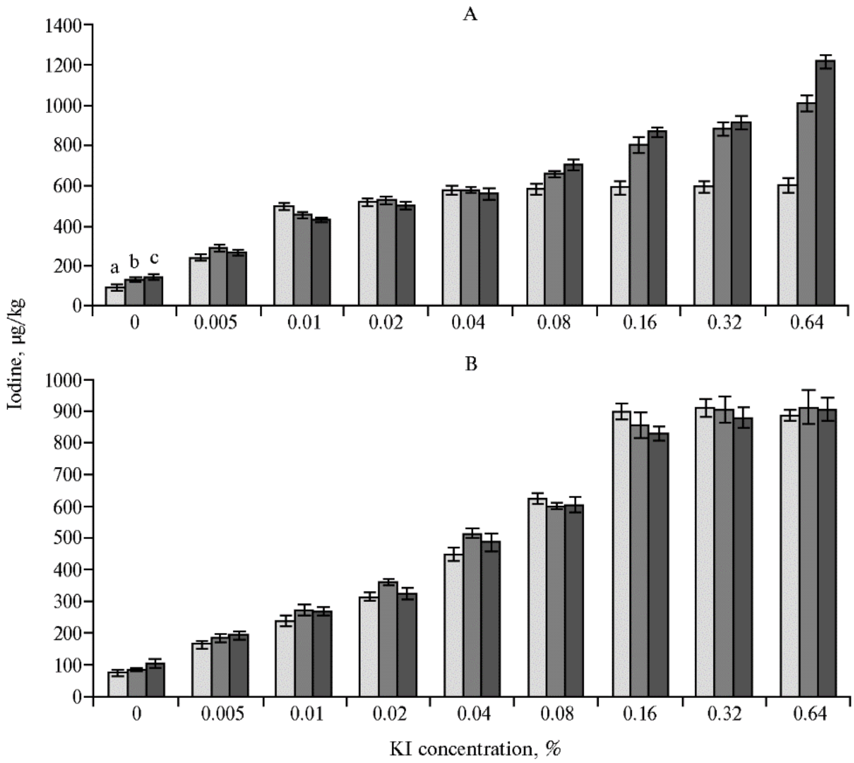

Fig. 1. Accumulation of iodine in the green mass of annuals (oat *Avena sativa* L. cv. Skakun, common vetch *Vicia sativa* L. cv. Vera) (A) **and perennial grasses (red clover *Trifolium pratense* L. cv. Orfei, meadow timothy *Phleum pratense* L. cv. Leningradskaya 204)** (B) **depending on the concentration of the KI solution and soil cultivation level:** a — medium cultivated, b — well cultivated, c — higly cultivated (n = 3, M±SEM, Menkovsky branch of the Agrophysical Research Institute, Gatchinsky District, Leningrad Province, 2019-2020).

On average, the accumulation of iodine in the aboveground plant biomass in the fertilized variants was 619 гg/kg for annual grasses and 557 μg/kg for perennial grasses. The lag of perennial grasses in this indicator by 9% was most likely due to the effect of "biological" dilution in the significantly superior yield of the latter. In the optimal options for the impact on crop productivity, the iodine content in the aboveground biomass of annual and perennial grasses was increased on average from 119 and 88 to 766 and 628 μg/kg, that is, by 6.4 and 7.1 times ($p \leq 0.05$).

The accumulation of nitrates in annual grasses largely depended on the soil cultivation level which determined soil nitrogen status. The content of N-NO_3^- in the arable layer of medium, well- and highly cultivated soil in the first decade of June was 19, 32 and 44 mg/kg, respectively, and increased in well- and highly cultivated soil by 34 and 122% ($p \leq 0.05$) (Fig. 2). Among perennial grasses, red clover which is little dependent on soil nitrogen, prevailed, and these herbs weakly respond to soil cultivation level.

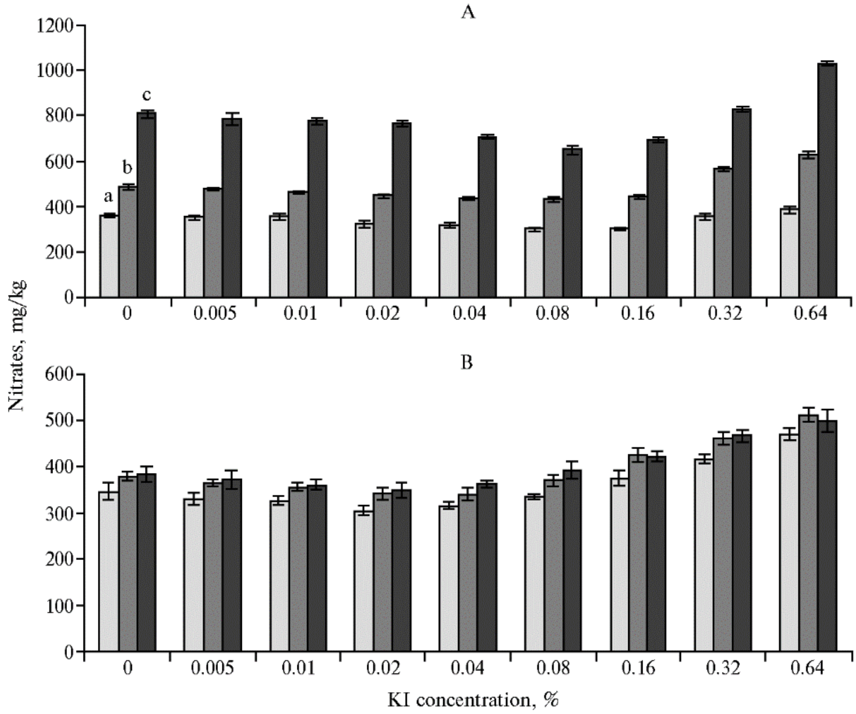

Fig. 2. Accumulation of nitrates in the green mass of annuals (oat *Avena sativa* L. cv. Skakun, common vetch *Vicia sativa* L. cv. Vera) (A) **and perennial grasses (red clover *Trifolium pratense* L. cv. Orfei, meadow timothy *Phleum pratense* L. cv. Leningradskaya 204)** (B) **depending on the concentration of the KI solution and soil cultivation level:** a — medium cultivated, b — well cultivated, c — higly cultivated (n = 3, M±SEM, Menkovsky branch of the Agrophysical Research Institute, Gatchinsky District, Leningrad Province, 2019-2020).

The effect of iodine on the accumulation of nitrates in grass biomass was much more complex. The important factors were the different availability of soil with nitrate nitrogen, which stimulated the biosynthetic effect in the area of low concentrations of the working solution and inhibited the reduction of nitrates in the cell in variants with high C_{KI} values [31, 32]. The maximum effect of reducing the accumulation of nitrates in the grass green mass occurred at C_{KI} 0.08% for annual grasses and C_{KI} 0.02% for perennial grasses. On average, for these treatments and crops, it reached 63 mg/kg (from 461 to 398 mg/kg), or 14%. In the test options that were optimal in terms of productivity, the average decrease in the content of nitrates reached 13% for annual grasses and 11% for perennial grasses ($p \leq 0.05$). Probably, largely for this reason, in the experiments of V.I. Panasin et al. [37] at KI concentrations up to 0.1%, there was a significant increase in the content of crude protein in the green mass of winter rapeseed.

An increase in the concentration of the working solution to the maximum values caused an increase in the accumulation of nitrates in the biomass of annuals by 128 mg/kg (from 552 to 680 mg/kg), or by 23%. In perennials, accumulation of nitrates increased by 123 mg/kg (from 370 to 493 mg/kg), or by 33% ($p \leq 0.05$), due to inhibition of reducing enzymes. Data comparable in terms of the relative increase in the content of nitrates under the influence of excess iodine (by 10-30%) were obtained for lettuce, wild carrot, and garden spinach [31, 32, 42].

In the second experiment, the nature of the influence of soil-agrochemical conditions and iodine foliar application on the qualitative parameters was largely similar (Fig. 3). Thus, the foliar use of 0.02% KI solution at the optimal time increased the iodine content in the green mass of annual grasses 5.1-fold (from 109 to 561 ґg/kg), of perennials 3.4-fold (from 99 to 333 ґg /kg). Unlike the perennials, annual grasses which are sensitive to the optimization of soil properties, reduced the iodine accumulation from medium level of cultivation to well- and highly cultivated soils due to “biological” dilution.

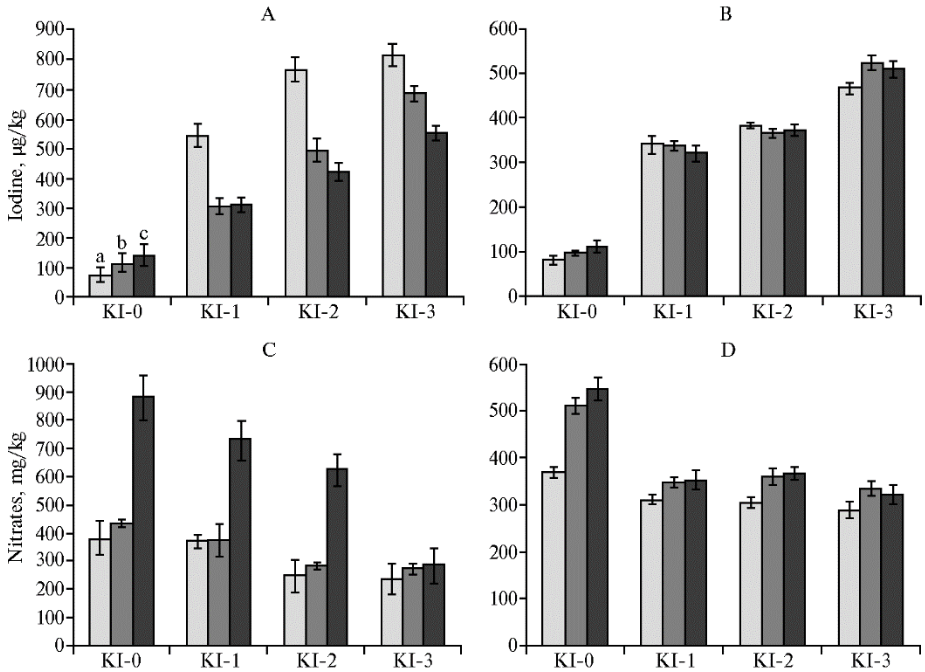

Fig. 3. Accumulation of iodine (A, B) **and nitrates** (C, D) **in the green mass of annuals (oat *Avena sativa* L. cv. Skakun, common vetch *Vicia sativa* L. cv. Vera)** (A, C) **and perennial grasses (red clover *Trifolium pratense* L. cv. Orfei, meadow timothy *Phleum pratense* L. cv. Leningradskaya 204)** (B, D) **depending on timing of KI application and soil cultivation level:** a — medium cultivated, b — well cultivated, c — higly cultivated (n = 6, M±SEM, Menkovsky branch of the Agrophysical Research Institute, Gatchinsky District, Leningrad Province, 2019-2020).

From the standpoint of providing better conditions for fixing iodine in the biomass, the later dates of foliar feeding were more effective. Justified in this regard was the two-time foliar spraying of grasses with a 0.02% KI solution, the positive effect of which on well- and highly cultivated soils was significantly higher. This option of using iodine microfertilizers also provided the best results for reducing the content of nitrates in grass biomass, especially on highly cultivated agro-soddy-podzolic soils. Their content in the green mass was reduced by 67% in annuals and by 41% in perennial grasses ($p \leq 0.05$), which is significantly higher than the values (18%) previously achieved both in our experiment on potatoes [34] and and on other crops [31, 32, 42].

Thus, the responsiveness of fodder grasses to iodine foliar apploction under geochemical iodine deficiency is determined by a combination of weather, climatic and soil agrochemical conditions with the biological properties of crops and depends on the timing of treatmens and the concentration of the KI working solution. For annual grasses, the optimal time for foliar application was the booting stage of oats, for perennial grasses, it was tillering of red clover and timothy grass. The yield of the annuals and perennials increased by 29% and 18%, respectively ($p \leq 0.05$). The optimal concentration of the working solution KI for annual grasses did not depend on the soil and agrochemical conditions and reached

0.16%. In perennial grasses, it was 0.04% on medium cultivated soil, 0.08% on well-cultivated soil, and 0.16% on highly cultivated soil. Due to the activation of the bioproduction due to the treatments, the yield of green mass increased ($p \leq 0.05$) in annual grasses by 3.69-9.38 t/ha (67-80%), in perennial grasses by 3.91-8.03 t/ha (22-30%). The content of iodine in the aboveground biomass of annuals and perennials increased ($p \leq 0.05$) on average from 119 and 88 to 766 and 628 mg/kg, that is, by 544 and 614%, while nitrates, on the contrary, decreased ($p \leq 0.05$) by 11-13%. The toxic effect of excess iodine, expressed in a decrease in crop productivity by 19% and an increase in the content of nitrates in the green mass by 23-33%, occurres at C_{KI} 0.32-0.64%. Legume components of grass mixtures, the common vetch and red clove are more sensitive to excess iodine.

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PROGRAM LEVEL OF AGROCENOSIS MANAGEMENT, TAKING INTO ACCOUNT THE IMPACT OF WEEDS ON CROPS

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Abstract

In modern crop production, a traditional paradigm of separate management of crops and weeds as part of single agrocenosis dominates. However, mineral fertilizers simultaneously stimulates the growth and development of crops and weeds, and herbicides suppress the growth of both cultivated plants and weeds. This leads to significant yield losses and waste of fertilizers and herbicides. The purpose of this study is to develop a theoretical basis for solving the problem of managing agrocenoses, which include the main crop and weeds. The solution of this problem is aimed at eliminating the limitations of the existing paradigm of separate management of crops and weeds in the agrocenosis. Previously, we have developed a theory of management of agro-technologies, in which the object of management is an agricultural crop without considering the role of weeds in the agrocenoses. In accordance with this theory, the crop management is carried out at strategic, program and real-time levels. In the presented work, for the first time, the problem of managing agrocenoses at the program level during one growing season is posed and solved. The essence of the approach lies in the development of the programs, which are sequences of technological operations for the application of mineral fertilizers, irrigation and herbicide treatments, providing a given crop yield with minimal expenditure of resources. To solve this problem, in the previously developed theory, mathematical models for crop parameters are modified to reflect the effect of herbicides. In addition, a model of the parameters of dominant weed species was introduced into the control task, in which, in addition to the doses of herbicide treatments, the effect of the doses of mineral fertilizers is reflected. The mathematical model for the soil environment, which takes into account the influence of the parameters of the state of the cultivated crop and weeds, has also undergone significant refinement. The problem is solved on the example of sowing spring wheat as part of an agrocenoses. The presence of several spring wheat phenological phases needs to transform the structure and parameters of the mathematical models used for all phenophases. This, in turn, needs to solve the problem of forming optimal programs for managing agrocenoses separately for each interphase period and combine the received private programs into a single program. As a method for solving the problem, the Pontryagin's maximum principle is used in combination with a dynamic programming scheme (from the end of the growing season to its beginning). The structural complexity of the control object, which is an agricultural field with agrocenoses, necessitates solving the problem of program control in three stages. At the first stage, a program is formed to change the parameters of the soil environment, which ensures the achievement of the required crop yield. At this stage, the effect of herbicide treatments on the state of crop sowing is not considered. At the second stage, a sequence of technological operations is found that provides the best approximation of soil parameters to the optimal program obtained at the first stage. Finally, at the third stage, the optimal sequence of herbicide treatments performed simultaneously with other technological operations is found. To consider the influence of these processing, the programs obtained at the first two stages are refined until the convergence of the solution of the entire problem is obtained.

Keywords: program control, agrocenoses, mineral fertilizers, herbicides, mathematical models, control algorithms

In modern crop production, the traditional paradigm of separate management of crops and weeds as part of one agrocenosis has long been established. The development of precision farming (PF) stimulates creation of an effective theory for agricultural technology management. But this mainly concerns the crops managing. Many provisions of modern theory have already been developed, from a general concept of control to algorithms for control at various time levels [1, 2]. According to this theory, it becomes possible to form control programs which are sequences of technological operations with the optimal level of fertilizer doses and irrigation rates. As for the management of weeds, the progress in the development of the theory is more modest, and to date, the optimal doses of herbicide treatments have not yet been scientifically substantiated. Note, the existing theoretical base for managing the state of agricultural crops does not take into account the fact that weeds are present in the agrocenosis, and the application of fertilizers and irrigation stimulate weed growth and development along with the crop. And vice versa, the treatment with herbicides inhibits not only weeds but also the main crop of agrocenosis. As a result, such separate management leads to significant crop losses, overspending of fertilizers and herbicides, and deterioration of environmental performance. The recent appearance of a sufficient number of publications on the joint application of fertilizers and herbicides shows that technological science is striving to eliminate the shortcomings of the existing separate management paradigm [3-5]. This poses new challenges for the science of managing agricultural technologies, forcing to consider a field with an agrocenosis as a single management object (MO).

According to the proposed concept of PF management, the overall task includes four levels of sub-tasks solved at different time scales [1]. At the top 1st level, crop rotation managing on an annual scale must be solved; at the 2nd level, implemented on a daily scale at one vegetation interval, the program control is solved. The tasks of two planning levels are decided in advance, out of real time. Tasks of the 3rd and 4th levels, where technological operations are directly formed, are implemented in a real time mode. Of all the above levels, the program level of control is the key one, since it is through it that the strategic tasks of management are connected with real-time tasks. At the program level of management, an optimal sequence of technological operations is planned to ensure the achievement of the desired result [2].

In accordance with the proposed concept of management at the program level, a field with an agricultural crop is an MO. However, the concept does not take into account the fact that in the same field as part of the agrocenosis, in addition to the main crop, annual and perennial weeds grow. They compete with the plants of the cultivated crop for moisture and nutrients, and crop losses from weed infestation can exceed 50%. Therefore, the optimal technological programs of operations in the considered vegetation interval should include not only fertilization and irrigation operations, but also herbicide treatments. Such programs should be formed given the fact that mineral nutrition stimulates the growth of both cultivated plants and weeds, and herbicides not only suppress the growth and development of weeds, but also act depressingly on cultivated plants.

The formation of a unified program of simultaneous application of mineral fertilizers and herbicide treatments, coordinated according to the state of the main crop and weeds, will avoid crop losses and cost overruns of mineral fertilizers and herbicides. In addition, optimization of fertilizer doses that meet the biological needs of the crop in nutrients activates metabolic processes, accelerates the inactivation of the incoming herbicide and increases the resistance of the protected plant to it. The protected crop, due to more intensive accumulation of organic mass, receives a significantly lower dose of herbicide per unit mass, that is, there is a decrease in the herbicide content in tissues during growth, and smaller amounts of the drug, given optimal metabolism, are inactivated faster. Optimal nutritional conditions also increase the overall biological competitiveness of the crop against weeds [3-5].

An analysis of foreign publications has shown that today only particular aspects and little interconnected tasks of managing the crop state have been developed, including models for estimating and predicting crop biomass [7, 9], principles of zonal management of nitrogen nutrition and risks [10-12, 18], general principles of agricultural production resource management with resource models [13-17, 19]. Thereof, it can be argued that a unified theoretical framework for managing agricultural technologies in PF has not yet been developed.

This is due to insufficient knowledge of agrocenosis as a single MO for which complex models of the relationship between the state of crops and weeds in sowing have not yet been created. A large review paper [20] discusses the nature and practice of using a full range of simulation models for ecology, biology and weed control, and the use of such models for information and decision support. As a rule, plant protection specialists proceed from a quantitative assessment of the population density and aggressiveness of the weeds. An important place is occupied by models that predict crop losses and thresholds for determining the methods and timing of weed control measures [21, 22]. Several approaches have been implemented based on the relationship between weed density in crops and yield loss, and it has been proven that this relationship is described by a rectangular hyperbola [23].

Weed biomass can be a reliable predictor of crop loss [24, 25]. The higher it is (regardless of the density of weeds in the agrocenosis), the more the crop yield decreases. However, accounting for weed biomass is time consuming and difficult to control in the field. Another problem in predicting crop losses from weed biomass is that there is no clear understanding of how much of this biomass should be considered. An example of predicting the impact of weeds and crop losses is the model proposed by M.J. Krop and J.T. Spitters [26] for sugar beet.

Adaptive changes in weeds are another factor that must be taken into account when analyzing the weed-crop system. With an increase in the competitiveness of weeds, the potential of agricultural crops will decrease. As a consequence, the accuracy of predictive crop loss models due to weeds will gradually decrease [27, 28]. Therefore, to correctly take into account the mutual influence of weeds and crops, periodic parameterization and recalibration of models is necessary.

In practice, solving the problem of crop losses from weeds requires knowledge of the species-specific, time-varying relationships between weeds and cultivated plant species, understanding the short-term and long-term consequences of the adopted tactics of protective measures [29, 30]. Without similarly interpreted multifactorial events, it is impossible to design protective programs that are effective, functional, and will not harm non-target organisms [31-34].

The need for decision support tools (DST) for practitioners is especially great. Such DST should combine models of weed population dynamics, economic efficiency of technology [35-37] and its impact on the environment [36, 37]. Such DST will allow practitioners to model new management options in local conditions, adapt sustainable management concepts to the characteristics of resident weeds [38-40], and compare the likely short-term outcomes of possible interventions. Some

DSTs model the short-term results of mechanical control tactics [31, 41-43]. DST tactics are most effective for chemical weed control [36, 37, 42, 44, 45]. These DSTs provide guidance to choose a number of aspects of herbicide application [31, 38, 46-49]. In addition, many solutions offer projections on financial interventions [31, 42, 46-49].

Decision support tools for preventive management serve two main purposes: first, they predict weed infestation and returns from different interventions over several years; second, they provide these forecasts within the scope of the respective intended target [40-44].

The analysis shows that, with all the breadth of covering the problem, the proposed tools do not include reasonable mathematical models and the choice of achievable management goals, optimality criteria and effective control algorithms to develop of technological operation programs that ensure management goals.

In the proposed work, for the first time, we pose and solve the problem of unified management of agrocenosis at the program level for one growing season. To solve it, in the previously developed theory of management of agricultural technologies, the mathematical models of the crop state are modified to reflect the effect of herbicides. In addition, a model for the parameters of the dominant species of weeds was used, in which, together with the dosage of herbicides, the influence of mineral fertilizers is considered. We have proposed a novel three-stage procedure for the program management of agrocenosis. At the first stage, a program is formed to change the soil conditions, ensuring the required crop yield. At this stage, the effect of herbicides on the crop sowing is not taken into account. At the second stage, a sequence of technological operations is found that provides the best approximation of soil parameters to the optimal program obtained at the first stage. Finally, at the third stage, the optimal sequence of herbicide treatments performed simultaneously with other technological operations is found.

The purpose of this work was to further develop the theory of programmed management for an agrocenosis with spring wheat crops as the MO to be controlled.

Materials and methods. The classical control theory with dynamic programming and the Pontryagin maximum principle [6] were used. According to this theory, the starting point for solving any control problem is the choice of an achievable goal. When we are dealing with agricultural technology, such a goal can only be to obtain a given crop yield at the end of the growing season. Any control task is based on the mathematical description of the MO. In the case under consideration, this is an agricultural field with an agrocenosis which includes a crop of spring wheat. The fundamental basis for solving problems of program control are mathematical models that describe the dynamics of the parameters of the state of the control object (MO). In addition to the mathematical model of the main crop, the MO should be supplemented by a model of weeds. Such models should reflect the influence of external uncontrolled disturbances, the influence of controlled factors on the MO parameters and take into account their interconnection through the soil environment.

To achieve the above management goal, it is necessary to select the most important goal-forming parameters of the crop sowing. The crop under consideration is characterized both by continual state parameters, which include the crop biomass and soil parameters, and by structural state parameters, including phenophases. For spring wheat, depending on the duration of the interval on a daily time scale t, these are at $t \in (0-7)$ s = 1, sowing; at $t \in (11-13)$ s = 2, seedlings (1st, 2nd, 3rd leaves); at $t \in (21-29)$ s = 3, tillering; at $t = 30$ s = 4, stem extension; at $t \in (31-32)$ s = 5, internode; $t = 37$ s = 6, flag leaf; at $t = 39$ s = 7, ligula; at

$t = 49$ s = 8, leaf sinus opening; at $t \in (51-59)$ s = 9, heading; at $t \in (61-69)$ s = 10, flowering; at $t \in (71-75)$ s = 11, milky ripeness; at $t \in (85-86)$ s = 12, wax ripeness; at $t \geq 86$ s = 13, full ripeness.

The entire growing season, depending on the structure of the sowing biomass, can be divided into two time intervals, from the 2nd to the 9th and from the 9th to the 13th phenophase. Fertilization, herbicide treatments and watering are carried out at fixed times of the onset of the following pre-selected phenological phases: s = 3 (tillering), s = 9 (earing), s = 10 (flowering), s = 11 (milky ripeness). This makes it necessary to break down the entire management interval into four intervals between the selected phenophases: 1st from tillering to heading (T_3, T_9), 2nd from heading to flowering (T_9, T_{10}), 3rd from flowering to milky ripeness (T_{10}, T_{11}), and 4th from milky ripeness to full ripeness (T_{11}, T_{13}).

For the first time interval (the phenophases from the 3rd to the 9th), the model for dynamics of crop biomass structure parameters has the form [1, 2]:

$$\begin{bmatrix} \dot{x}_{1m} \\ \dot{x}_{2m} \end{bmatrix} = \begin{bmatrix} a_{11} & a_{12} \\ a_{21} & a_{22} \end{bmatrix}_m \begin{bmatrix} x(t)_{1m} \\ x(t)_{2m} \end{bmatrix} + \begin{bmatrix} b_{11} & b_{12} & b_{13} & b_{14} \\ b_{21} & b_{22} & b_{23} & b_{24} \end{bmatrix}_m \begin{bmatrix} v_N(t) \\ v_K(t) \\ v_P(t) \\ v_{Mg}(t) \\ v_5(t) \end{bmatrix} + \begin{bmatrix} c_{11} & c_{12} & c_{13} \\ c_{21} & c_{22} & c_{23} \end{bmatrix}_m \begin{bmatrix} f_1(t) \\ f_2(t) \\ f_3(t) \end{bmatrix} - \begin{bmatrix} d_{11} & d_{12} \\ d_{21} & d_{22} \end{bmatrix}_m \begin{bmatrix} g_1(t) \\ g_2(t) \end{bmatrix}_m, \; t \in (T_3, T_9), \quad (1)$$

where $\underline{x_{1m}}$ is the average planting biomass density (yield), t · ha^{-1} over the area of the field; $\underline{x_{2m}}$ is the density of the wet mass of crops averaged over the area of the field, c · ha^{-1}; external disturbances in both blocks are $\underline{f_1}$, the average daily air temperature, °C; f_2, the average daily radiation level, W · (m^2 · h)$^{-1}$; $\underline{f_3}$, the average daily precipitation intensity, mm. Chemical parameters of the soil are v_N, the nitrogen content, kg · ha^{-1}; $\underline{v_K}$, the potassium content, kg · ha^{-1}; v_P, the phosphorus content, kg · ha^{-1}; $\underline{v_{Mg}}$, the magnesium content, kg · ha^{-1}; v_5 is the moisture reserve in the soil, mm; $g_{1m}(t)$, $g_{2m}(t)$ are doses of a herbicide, g · ha^{-1}. Because of research and methodological reasons of the work, we do not disclose the types of herbicides here, since the approach we develop is applicable to any herbicides.

For further use, it is convenient to represent the model (1) in the canonical symbolic vector-matrix form, where all variables are combined into vectors, and parameters into the corresponding matrices:

$$\dot{X}_m = A_m X_m(t) + B_m V(t) + C_m F(t) - D_m G_m(t). \quad (2)$$

For intervals from the second, the *(T_9, T_{10}), (T_{10}, T_{11}) and (T_{11}, T_{13})* the models of the dynamics of crop biomass structure parameters have a general form and differ only in the values of the parameters [1, 2]:

$$\begin{bmatrix} \dot{x}_{1u} \\ \dot{x}_{2u} \\ \dot{x}_{3u} \end{bmatrix}_j = \begin{bmatrix} a_{11} & a_{12} & a_{13} \\ a_{21} & a_{22} & a_{23} \\ a_{31} & a_{32} & a_{33} \end{bmatrix}_j \begin{bmatrix} x(t)_{1u} \\ x(t)_{2u} \\ x(t)_{3u} \end{bmatrix}_j + \begin{bmatrix} b_{11} & b_{12} & b_{13} & b_{14} & b_{15} \\ b_{21} & b_{22} & b_{23} & b_{24} & b_{25} \\ b_{31} & b_{32} & b_{33} & b_{34} & b_{35} \end{bmatrix}_j \begin{bmatrix} v_N(t) \\ v_K(t) \\ v_P(t) \\ v_{Mg}(t) \\ v_5(t) \end{bmatrix}_j + \begin{bmatrix} c_{11} & c_{12} & c_{13} \\ c_{21} & c_{22} & c_{23} \\ c_{31} & c_{32} & c_{33} \end{bmatrix}_j \begin{bmatrix} f_1(t) \\ f_2(t) \\ f_3(t) \end{bmatrix} - \begin{bmatrix} d_{11} & d_{12} \\ d_{21} & d_{22} \\ d_{31} & d_{32} \end{bmatrix}_j \begin{bmatrix} g_1(t) \\ g_2(t) \end{bmatrix}_j, \quad [3]$$

$$j = 1, t \in (T_9, T_{10}); \; j = 2, \; t \in (T_{10}, T_{11}), \; j = 3, \; t \in (T_{11}, T_{13}).$$

In this model, the x_{1u} is the average density of the crop biomass over the area of the field, c · ha^{-1}; x_{2u} is the density of the wet mass of crops averaged over the area of the field, c · ha^{-1}; x_{3u} is the density of the mass of ears (yield) averaged over the area of the field, c · ha^{-1}; external disturbances in both blocks are f_1- average daily air temperature, °C; f_2 — average daily radiation level, W · (m^2 · h)$^{-1}$; f_3 is the average daily precipitation intensity, mm; chemical parameters of the soil are : v_N - nitrogen content in the soil, kg · ha^{-1}; v_K is the content of potassium in the soil, kg · ha^{-1}; v_P is the content of phosphorus in the soil, kg · ha^{-1}; v_{Mg} is the content of magnesium in the soil, kg · ha^{-1}; v_5 is the moisture content in the soil, mm; $g_{1u}(t)$, $g_{2u}(t)$ are doses of herbicide treatment, g · ha^{-1}; j = 1, 2, 3 are the numbers of control intervals after the heading phase.

The canonical symbolic vector-matrix form of the model has the following form [3]:

$$\dot{X}_{uj} = A_{uj} X_{uj}(t) + B_{uj} V_{uj}(t) + C_{uj} F(t) - D_{uj} G_{uj}(t). \quad (4)$$

As mentioned above, in addition to the model (4), to solve the problem, a dynamic model of the biomass of the dominant weed species is required, the vector-matrix form of which has the form

$$\dot{S}_j = A_{sj} S_j(T) + B_s V_j(T) - B_{gj} G_j(t) + C_{sj} F(t), \quad (5)$$

Where $S^T = [s_1 \ s_2]$ is the biomass vector of the dominant weed species, T is the transposition index of the vector or matrix,

$$A_s = \begin{bmatrix} a_{11} & 0 \\ 0 & a_{22} \end{bmatrix}_s, \ B_s = \begin{bmatrix} b_{11} & b_{12} & b_{13} & b_{14} \\ b_{21} & b_{22} & b_{23} & b_{24} \end{bmatrix}_s, \ B_g = \begin{bmatrix} b_{11} & 0 \\ 0 & b_{22} \end{bmatrix}_g, \ C_s = \begin{bmatrix} c_{11} & c_{12} & c_{13} & c_{14} \\ c_{21} & c_{22} & c_{23} & c_{24} \end{bmatrix}_s$$

are matrices of model parameters.

The model (5) includes the states of two dominant weed species. For other conditions, their number and types may be different, which only refines the structure of the algorithms, but does not change the general approach to solving the problem.

Models (2), (4) and (5) represent the main block of MO state parameters. In addition to this block, the MO contains a block of control transfer, which is the soil environment (SE). It is through this block that crop plants and weeds compete for nutrients and moisture.

The model of dynamics of soil state parameters for phenophases 3 to 9 takes the following form [1]:

$$\begin{bmatrix} \dot{v}_N \\ \dot{v}_K \\ \dot{v}_P \\ \dot{v}_{Mg} \\ \dot{v}_5 \end{bmatrix}_{3,9} = \begin{bmatrix} a_{11} & 0 & 0 & 0 & a_{15} \\ 0 & a_{22} & 0 & 0 & a_{25} \\ 0 & 0 & a_{33} & 0 & a_{35} \\ 0 & 0 & 0 & a_{44} & a_{45} \\ 0 & 0 & 0 & 0 & a_{55} \end{bmatrix}_{3,9} \begin{bmatrix} v_N \\ v_K \\ v_P \\ v_{Mg} \\ v_5 \end{bmatrix}_{3,9} + \begin{bmatrix} 1 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 1 \end{bmatrix}_{3,9} \begin{bmatrix} d_N(t) \\ d_K(t) \\ d_P(t) \\ d_{Mg}(t) \\ d_W(t) \end{bmatrix}_{3,9} +$$
$$+ \begin{bmatrix} 0 & 0 & c_{13} \\ 0 & 0 & c_{23} \\ 0 & 0 & c_{33} \\ 0 & 0 & c_{43} \\ c_{51} & c_{52} & 1 \end{bmatrix}_{3,9} \begin{bmatrix} f_1(t) \\ f_2(t) \\ f_3(t) \end{bmatrix} - \begin{bmatrix} m_{11} & 0 \\ m_{21} & 0 \\ m_{31} & 0 \\ m_{41} & 0 \\ m_{51} & m_{52} \end{bmatrix}_{3,9} \begin{bmatrix} x_{1m}(t) \\ x_{2m}(t) \end{bmatrix} - \begin{bmatrix} p_{11} & p_{12} \\ p_{21} & p_{22} \\ p_{31} & p_{32} \\ p_{41} & p_{42} \\ p_{51} & p_{52} \end{bmatrix}_{3,9} S(t), \quad (6)$$

or

$$\dot{V}_{3,9} = A_{3,9} V(t) + B_{3,9} D(T_3, T_9) + C_{3,9} F(t) - M_{3,9} X_m(t) - P_{3,9} S(t). \quad (7)$$

Models of the dynamics of soil state parameters for the intervals (T_9, T_{10}), (T_{10}, T_{11}) and (T_{11}, T_{13}) have the same form, differing only in the values of the parameters:

$$\begin{bmatrix} \dot{v}_N \\ \dot{v}_K \\ \dot{v}_P \\ \dot{v}_{Mg} \\ \dot{v}_5 \end{bmatrix}_j = \begin{bmatrix} a_{11} & 0 & 0 & 0 & a_{15} \\ 0 & a_{22} & 0 & 0 & a_{25} \\ 0 & 0 & a_{33} & 0 & a_{35} \\ 0 & 0 & 0 & a_{44} & a_{45} \\ 0 & 0 & 0 & 0 & a_{55} \end{bmatrix}_j \begin{bmatrix} v_N \\ v_K \\ v_P \\ v_{Mg} \\ v_5 \end{bmatrix}_j + \begin{bmatrix} 1 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 1 \end{bmatrix}_j \begin{bmatrix} d_N(t) \\ d_K(t) \\ d_P(t) \\ d_{Mg}(t) \\ d_W(t) \end{bmatrix}_j + \begin{bmatrix} 0 & 0 & c_{13} \\ 0 & 0 & c_{23} \\ 0 & 0 & c_{33} \\ 0 & 0 & c_{43} \\ c_{51} & c_{52} & 1 \end{bmatrix}_j \begin{bmatrix} f_1(t) \\ f_2(t) \\ f_3(t) \end{bmatrix} - \begin{bmatrix} m_{11} & 0 & m_{13} \\ m_{21} & 0 & m_{23} \\ m_{31} & 0 & m_{33} \\ m_{41} & 0 & m_{43} \\ m_{51} & m_{52} & 0 \end{bmatrix}_j \begin{bmatrix} x_{1u}(t) \\ x_{2u}(t) \\ x_{3u}(t) \end{bmatrix}_j - \begin{bmatrix} p_{11} & p_{12} \\ p_{21} & p_{22} \\ p_{31} & p_{32} \\ p_{41} & p_{42} \\ p_{51} & p_{52} \end{bmatrix}_j S(t), \quad (8)$$

where $d_P(t)$, $d_K(t)$, $d_N(t)$, $d_W(t)$ are the doses of nutrients (phosphorus P, potassium K, ameliorant Ca, nitrogen N and magnesium Mg, kg · ha^{-1}, respectively) and irrigation rate, mm; a_{11}–a_{33}, b_2–b_3, c_1–c_3 are model parameters estimated from experimental data; t is days.

Model (8) in compact symbolic vector-matrix form is

$$\dot{V}_j = A_j V_j(t) + B_j D_j(t) + C_j F(t) - M_j X_j(t) - P_j S(t). \quad (9)$$

The complex multidimensional structure of the MO, including models (2), (4), (5) of the main block of state parameters and blocks of control transfer (7), (9), needs to solve the program control problem in two stages [1, 2]. At the first stage, there is a program for the potential development of crop sowing throughout the growing season, which ensures the expected management goal. In this case, the influence of weeds is not taken into account, and the parameters of the state of the SE are considered as control variables without taking into account technological limitations. The control program obtained in this way serves as a guide to form a sequence of technological operations, including fertilization, watering and herbicide treatments.

Therefore, at the second stage, a sequence of technological operations is found, which should ensure the minimum deviation of the SE parameters from the optimal program obtained at the first stage. Such a decomposition of the program control greatly simplifies the synthesis of optimal control programs. In addition, the optimization results obtained at the first stage are of independent interest as a characteristic of the potential level of crop yield.

In accordance with the dynamic programming scheme [6], the task of the first stage is solved from the end of the growing season to its beginning. In this case, the goal of management is to obtain a given crop yield at the end of the growing season under the following conditions: achieving the required structure of the entire biological crop (namely, the required quantitative ratio between grain and straw), the required grain moisture content, as well as reducing the biomass of weeds in the agrocenosis to the specified level.

In the indicated state parameters, the control goal for the specified vegetation interval formally looks like this:

$$x_{1u}(T_{13}) \geq 2{,}1U^*, \; x_{2u}(T_{13}) \leq 0{,}15U^*, \; x_{3u}(T_{13}) \geq U^*, \; S_{uj}(T_{13}) \leq S^*_{uj},$$

where $U^*(T_{13})$ is given yield, c · ha^{-1}, S^*_{uj} is given weed biomass.

The optimality criterion for the non-vegetation period from the 9th to the

13th phenophase, which meets the goal, has the following form

$$\begin{aligned} J_{uj}(T_{13}) = [X_{uj}(T_{13}) - X^*_{uj}(T_{13})]^T G_u [X_{uj}(T_{13}) - X^*_{uj}(T_{13})] + \\ +[S_{uj}(T_{13})] - S^*_{uj}(T_{13})]^T Q [S_{uj}(T_{13})] - S^*_{uj}(T_{13})], \end{aligned} \quad (10)$$

where $X^{*T} = [2{,}1U^* \; 0{,}15U^* \; U^*]$ is a vector which components are total biomass, fresh weight, grain weight (yield);

$G_u = \begin{bmatrix} g_{11} & 0 & 0 \\ 0 & g_{22} & 0 \\ 0 & 0 & g_{33} \end{bmatrix}_u$ is weight matrix of mass and quality components of the criterion,

$Q = \begin{bmatrix} q_1 & 0 \\ 0 & q_2 \end{bmatrix}$ is weight matrix of weed biomass components,

$j = 0,1,2,3$ – control interval indices.

To form optimal programs for all control intervals, the Pontryagin's maximum principle [6] is used. In accordance with this method, the Hamiltonian of the system, which includes the models (4), (5) and the criterion (10), has the following form:

$$\begin{aligned} H_{uj}(t) = \Psi^T{}_{1,uj}[A_{uj} X_{uj}(t) + B_{uj} V_{uj}(t) + C_{uj} F(t) - D_{uj} G_{uj}(t)] + \\ +\Psi^T{}_{2,uj}[A_s S_{uj}(T) + B_s V_{uj}(T) - B_g G_j(t) + C_s F(T)]. \end{aligned} \quad (11)$$

and models of conjugate variables are

$$\dot{\Psi}_{1,uj=3,i} = -\frac{\partial H_{uj=3,i}}{\partial X_{1,uj=3,i}} = -A^T_{uj=3,i} \Psi_{1,uj=3,i}, \quad t \in (T_{11}, T_{13}), \; \Psi_{1,uj=3,i}(T_{13}) = [X_{uj}(T_{13}) - X^*_{uj}], \quad (12)$$

$$\dot{\Psi}_{2,uj=3,i} = -\frac{\partial H_{uj=3,i}}{\partial S_{1,uj=3,i}} = -A^T_{sj=3,i} \Psi_{2,uj=3,i}, \quad t \in (T_{11}, T_{13}), \; \Psi_{2,uj=3,i}(T_{13}) = 2[S_{uj}(T_{13})] - S^*_{uj}]. \quad (13)$$

The algorithm for the formation of an optimal program for changing the parameters of the soil environment, which ensures the management goal, includes the following iterative procedures:

1) formation of optimal programs for changing the parameters of the substation

$$\begin{aligned} V^*_{uj,i+1}(t) = V^*_{uj,i}(t) - \Delta_i GR_{uj,i}(t), \\ GR_{uj,i}(t) = \frac{\partial H}{\partial V_{uj,i}}(t) = B^T{}_{uj} \Psi_{1uj,i}(t) + B^T{}_s \Psi_{2,uj,i}(t). \end{aligned} \quad (14)$$

2) search for initial conditions at the boundaries of phenophases:

$$X^*_{uj,i+1}(T_{11}) = X^*_{uj,i}(T_{11}) - \Delta_i \Psi_{1,uj,i}(T_{11}), \quad (15)$$

$$S^*_{uj,i+1}(T_{11}) = S^*_{uj,i}(T_{11}) - \Delta_i \Psi_{2,uj,i}(T_{11}). \quad (16)$$

As a result of solving the task of the first stage, an optimal program for changing the vector of SE parameters V*(t) is formed, which consists of separate pieces at four control intervals between the selected phenophases. This program corresponds to the program for changing the vector of sowing state parameters X*(t) and the program for changing the vector of weed biomass parameters S*(t) at these control intervals.

The achievable management goal at the second stage of the general solution is to ensure the closest approximation to the optimal programs for the content of nutrients and moisture in the soil obtained at the first stage of the forecast by independently choosing the doses of fertilizers and watering. Fertilization and irrigation are carried out at fixed times of the onset of the following phenological phases: s = 3 (tillering), s = 9 (heading), s = 10 (flowering), s = 11 (milky ripeness).

As for the first stage, the problem is solved separately for each control interval, but in the forward direction - from the beginning of the growing season to its end.

Particular optimality criteria for each j-th control interval have the same form:

$$J_j = \int_{T_{1j}}^{T_{2j}} [(\mathrm{V}_j^*(t)-\mathrm{V}_j(t))^{\mathrm{T}}\,\mathrm{G}_j(\mathrm{V}_j^*(t)-\mathrm{V}_j(t))+\mathrm{C}_\mathrm{D}\,D_j(t)]dt,\ j=0,1,2,3, \tag{17}$$

$$\mathrm{G}_j = \begin{bmatrix} g_1 & 0 & 0 & 0 & 0 \\ 0 & g_2 & 0 & 0 & 0 \\ 0 & 0 & g_3 & 0 & 0 \\ 0 & 0 & 0 & g_4 & 0 \\ 0 & 0 & 0 & 0 & g_5 \end{bmatrix}_j$$ is weight matrix, $\mathrm{C_D}$ is vector of "prices" per dose unit.

The criterion (17) is formed by means of the model of soil state parameters (9):

$$\dot{\mathrm{V}}_j = \mathrm{A}_j\mathrm{V}_j(t)+\mathrm{B}_j\,D_j(t)+\mathrm{C}_j\,\mathrm{F}(t)-\mathrm{M}_j\,\mathrm{X}_j^*(t)-\mathrm{P}_j\,\mathrm{S}_j^*(t),$$

where $\mathrm{X}^*j(t)$, $\mathrm{S}^*j(t)$ are the optimal programs for the state parameters of crops and weeds obtained at the first stage.

The Hamiltonians for all control intervals are the same:

$$\begin{aligned} H_j &= [(\mathrm{V}_j^*(t)-\mathrm{V}_j(t))^{\mathrm{T}}\,\mathrm{G}_j(\mathrm{V}_j^*(t)-\mathrm{V}_j(t))+\mathrm{C}_\mathrm{D}\,D_j(t)]+ \\ &+\Psi_j^{\mathrm{T}}[\mathrm{A}_j\mathrm{V}_j(t)+\mathrm{B}_j\,D_j(t)+\mathrm{C}_j\,\mathrm{F}(t)-\mathrm{M}_j\,\mathrm{X}_j^*(t)-\mathrm{P}_j\,\mathrm{S}_j^*(t)], \end{aligned} \tag{18}$$

where Ψ_j^{T} are vectors of linked variables for j-th control intervals.

The linked variable models are as follows:

$$\dot{\Psi}_j(t) = -\frac{\partial \mathrm{H}_o(t)}{\partial V_j} = -[2\mathrm{G}_j((V_j^*(t)\text{-}V_j(t))+\tilde{\mathrm{A}}_j^{\mathrm{T}}\,\Psi_j(t)],\ t\in(T_{2j},T_{1j}),\ \Psi_j(T_{2j})=0 \tag{19}$$

The algorithm for generating sequences of fertilizer application doses (optimal control programs) includes an iterative procedure for sequentially searching for successive approximations of fertilizer and irrigation dose vectors:

$$D_{i+1}^*(T_{1j}) = D_i^*(T_{1j}) - \Delta_i^*\frac{\partial H_j(T_{1j})}{\partial D_i(T_{1j})}, \tag{20}$$

$$D_{i+1}^*(T_{1j}) = D_i^*(T_{1j}) - \Delta_i^*(C_D+\tilde{\mathrm{B}}_j^{\mathrm{T}}\,\Psi_{i,j}(T_{1j})),\quad \text{if}\quad \mathrm{D}_{i+1}^*(T_{1j})\in\Omega_j;$$

$$D_{i+1}^*(T_{1j}) = D_i^*(T_{1j}),\quad \text{if}\quad D_{i+1}^*(T_{1j})\notin\Omega_{T_{1j}}.$$

As a result of solving the task at the second stage, sequences of doses of fertilizer application and irrigation are formed over all management intervals $D^*j(T_1j)$, j = 0.1,2,3, which correspond to programs for changing the parameters of the soil environment $\mathrm{V}^*j(t)$, parameters sowing conditions $\mathrm{X}^*j(t)$ and weed biomass parameters $\mathrm{S}^*j(t)$.

The found optimal sequence of technological operations (program) does not yet take into account the direct effect of herbicides on the state of crop sowing in accordance with the models (2), (4). At the beginning of the procedure for the formation of a control program, such an effect cannot be taken into account, since the doses of treatments are not known a priori. Therefore, it is necessary to introduce one more external optimization cycle, in which such an influence is taken into account. To do this, it is necessary to close the entire procedure for the formation of an optimal control program for doses of herbicide treatment.

Models (4), (5) are used to solve the task:

$$\dot{\mathrm{X}}_{uj} = \mathrm{A}_{uj}\,\mathrm{X}_{uj}(t)+\mathrm{B}_{uj}\,\mathrm{V}_j^*(t)+\mathrm{C}_{uj}\,\mathrm{F}(t)-\mathrm{D}_{uj}\,G_{uj}(t),$$

$$\dot{S}_j = A_s S_j(t) + B_s V_j^*(t) - B_g G_j(t) + C_s F(t),$$

where $V^*j(t)$ is the optimal program for changing the parameters of the soil environment, obtained at the second stage.

The goal of management at this stage is to select such doses of herbicide treatments at all management intervals that provide the best approximation of predictive programs for changing the parameters of the state of crops and parameters of weed biomass to the optimal programs found at the second stage.

This goal corresponds to the following optimality criteria for individual control intervals:

$$J_j = \int_{T_{1j}}^{T_{2j}} [(X_j^*(t) - X_j(t))^T G_{1j}(X_j^*(t) - X_j(t)) + (S_j^*(t) - S_j(t))^T G_{2j}(S_j^*(t) - S_j(t))]dt, \quad (21)$$

$$j = 0,1,2,3$$

The algorithm for generating herbicide treatment programs is the following iterative procedure:

$$G_{j,i+1}^*(T_{1j}) = G_{j,i}^*(T_{1j}) - \Delta_i \, GR_{j,i}(T_{1j}),$$
$$GR_{j,i}(T_{1j}) = \frac{\partial H_{j,i}}{\partial G_{ji}}(T_{1j}) = D_j \Psi_{1j,i}(T_{1j}) + B_s \Psi_{2j,i}(T_{1j}). \quad (22)$$

At the first stage, the formation of fertilizer application programs was carried out without taking into account herbicide treatment programs. Given that fertilization and herbicide treatments are carried out simultaneously, fertilizer application programs need to be adjusted for herbicide application programs. For this, three global steps are introduced into the algorithm.

Step 1. The global cyclic variable k = 1 is accepted. The program of herbicide treatments $G^*(T_{1j})$ for all management intervals is substituted into the interval models, solving them from the beginning $j = 0$ to the end $j = 3$:

$$\dot{X}_{uj} = A_{uj} X_{uj}(t) + B_{uj} V_j^*(t) + C_{uj} F(t) - D_{uj} G_{uj}(t), \; t \in (T_{1J}, T_{2J}), \quad (23)$$

$$\dot{S}_j = A_s S_j(t) + B_s V_j^*(T) - B_g G_j(t) + C_s F(T), \; t \in (T_{1J}, T_{2J}), \quad (24)$$

in this case, the final solutions on the current interval are taken as initial ones for subsequent intervals: $X_0(T_{1j+1}) = X(T_{2j})$, $S_0(T_{1j+1}) = S(T_{2j})$. On the initial interval, the initial conditions common to the entire task are accepted: $X_0(T_{1j=0})$, $S_0(T_{1j=0})$. For the control interval, a global cyclic variable k = 1, $j = 3$ is taken, the criterion is calculated at the end of the growing season:

$$J_{k=1}(T_{13}) = [X_{uj=3}(T_{13}) - X_{uj=3}^*(T_{13})]^T G_u [X_{uj=3}(T_{13}) - X_{uj=3}^*(T_{13})] +$$
$$+ [S_{uj=3}(T_{13})] - S_{uj=3}^*(T_{13})]^T Q [S_{uj=3}(T_{13})] - S_{uj=3}^*(T_{13})]. \quad (25)$$

Step 2. If the criterion $J_{k=1}(T_{13})$ is less than the given value Δ, then STOP, otherwise the solutions of the models (4), (5) are transferred to point 1 of the first stage, and all stages of the task are repeated until obtaining a new criterion $J_{k=2}(T_{13})$.

Step 3. If the criterion $J_{k=2}(T_{13})$ is less than the criterion $J_{k=1}(T_{13})$, then the solutions of the models (4), (5) must be transferred to point 1 of the first stage, otherwise STOP, and decisions are made for the previous criterion $J_{k=1}(T_{13})$.

Results. The result of this work is the proposed algorithm for programmatic control of the state of agrocenosis with the sowing of spring wheat. This algorithm embodies a new theory of agrocenosis management and is implemented in a new specialized software product. The novelty and complexity of the algorithm requires its approbation on experimental data, which should reflect the possibility of using the obtained results in practice.

At its core, approbation consists in establishing the fact of the stability of the control algorithm and its convergence to the minimum of the chosen optimality criterion. These conditions can be obtained only with the qualitative identification of all mathematical models used in the problem. It was carried out according to experimental data obtained for 2015-2021 at the Menkovsky branch of the Agrophysical Research Institute (Leningrad Province).

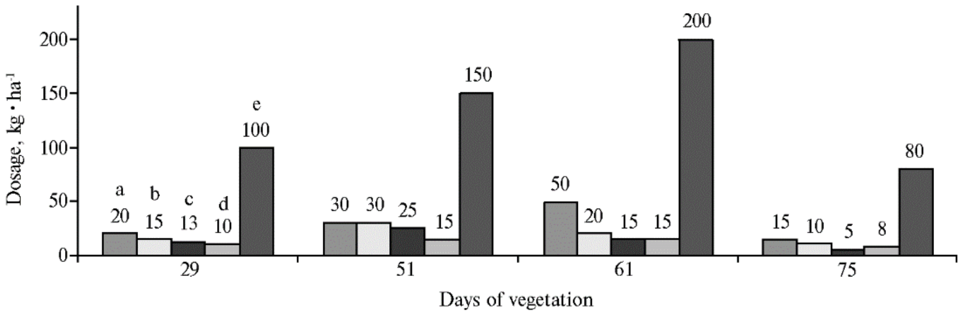
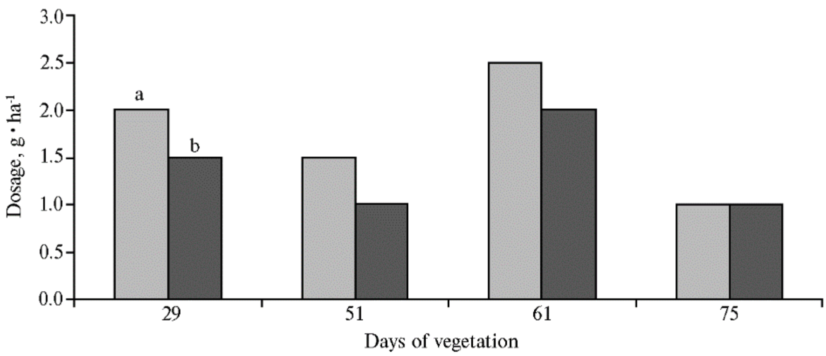


Fig. 1. The optimal program for the unified management of agrocenosis in terms of doses of mineral fertilizers and irrigation: a, b, c, d — doses of nitrogen, potassium, phosphorus and magnesium, respectively; e — irrigation rate, t • ha^{-1}.

The diagrams (Fig. 1, 2) show the results of optimization of agrocenosis management programs, including the sequence of doses of mineral fertilizers, irrigation and herbicide treatments. The program is focused on obtaining a given grain yield of 30 c/ha.



Fog. 2. Optimal program for unified management of agrocenosis by doses of herbicide treatments: a— herbicide 1 application rate, b — herbicide 2 application rate.

Here, technological operations were carried out at the onset of phenophases: t = 29 days (tillering), t = 51 days (heading), t = 61 days (flowering), t = 75 days (milky ripeness).

These programs correspond to the predicted dynamics of parameters of the biomass of spring wheat and two dominant weed species (Fig. 3, 4).

The given optimal control programs were obtained for 3 iterations of the global cycle of the algorithm, which corresponded to the following values of the optimality criterion [10] for the final sowing phenophase: iteration 1 — $J_u = 32$ $(c/ha)^2$, iteration 2 — $J_u = 14$ $(c/ha)^2$, iteration 3 — $\underline{J_u} = 6$ $(c/ha)^2$. Note that all the optimality criteria used are quadratic functions, so the dimension of their productivity is taken in the square. The decrease of the optimality criterion over iterations of the global cycle of the algorithm proves its stability and successive approximation to the minimum of the optimality criterion.

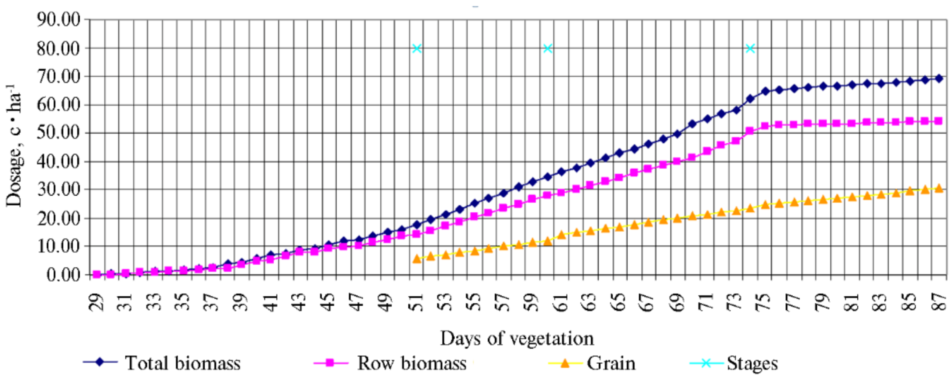


Fig. 3. Dynamics of biomass parameters of spring wheat sowing under the optimal program of unified management of agrocenosis.

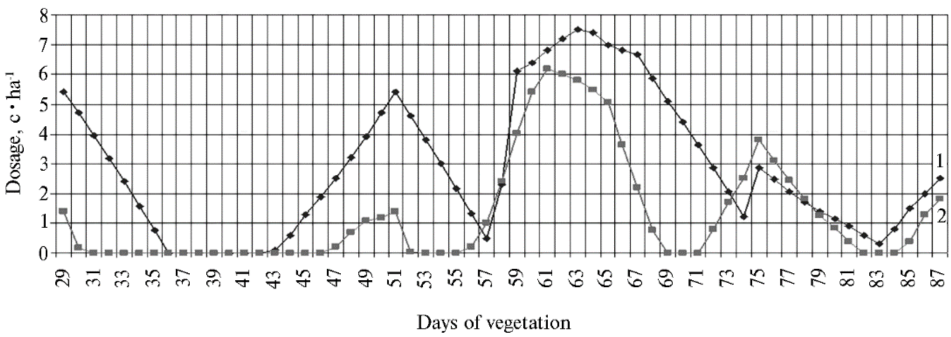


Fig. 4. Dynamics of biomass parameters of the dominant weed species under the optimal program of unified agrocenosis management: 1 — weed 1, 2 — weed 2.

As can be seen from the presented graph (see Fig. 3), the optimal program for the simultaneous application of fertilizers, watering and herbicide treatments ensures smooth growth and development of spring wheat sowing from germination to a given yield of 30 c/ha. The next graph (see Fig. 4) shows that weeds respond more dynamically to the technological operations of the optimal program, where stimulation with mineral fertilizers and irrigation causes an increase in weed biomass, which is suppressed by doses of herbicide treatments. At the same time, a general trend towards a decrease in weed biomass by the end of the growing season is manifested in the agrocenosis.

Based on the results of approbation of the problem, it can be argued that the proposed algorithm and specialized software package have characteristics sufficient to use this development as a means of intellectual support for an agronomist.

The algorithm and software package developed by us correspond to the concept of preventive management, which is considered as the main promising approach in measures to protect crops from weeds [20].

A number of models have been presented in the literature that quantify with some confidence the likelihood of outcomes for informed decision making, compare different management practices, and select options with the greatest long-term impact on the target group for agricultural units [20]. Selected examples provide an overview of models describing the distribution of weeds in crops, the use of such models in key areas of crop management, the quantitative findings and pragmatic results [20]. However, it is important to recognize that only few models (including ever developed decision support tools) have been widely applied to real pest management problems [38-41]. The main reasons cited are that practitioners consider the models to be inappropriate for local conditions and do not have the time to study typical operating procedures; models do not take into account changes in the structure and number of weed populations, in the economics of crop production, and software standards are not sufficiently supported [50-53].

As can be seen from the analysis of available publications, all models known so far are designed for individual systems in agriculture and crop production (cultivated plants—weeds, cultivated plants—fertilizers, weeds—herbicides). We have set and solved a fundamentally new problem and developed a program for the unified management of agrocenosis (the system of cultivated plants—weeds—fertilizers—-herbicides), which combines the listed particular tasks. The novelty of our invention is confirmed by the patent of the Russian Federation No. 2772889 "Method for the simultaneous differential application of liquid mineral fertilizers and herbicides and a device for its implementation" (dated May 26, 2022). The results of approbation showed the possibility of using this development in farm conditions.

Thus, we have proposed a new theory of programmed control of agrocenosis, focused on the implementation of the technological idea of the joint application of mineral fertilizers and herbicide treatments. It includes new mathematical models of parameters of the state of cultivated crops and weeds as part of agrocenosis, as well as new algorithms for the formation of optimal management programs. The algorithms are a four-stage sequential procedure which includes the creation of a program for the crop sowing development, the formation of a set of technological operations without herbicide treatments and a program with herbicide treatments carried out together with fertilization and irrigation, and, finally, the correction of programs for technological operations according to the herbicide treatments program. The proposed algorithm for optimal control programs avoids large dimensionality and complexity of the overall control task, ensures stability, a given crop yield and minimizes the weed biomass. The results obtained are a significant contribution to modern digitalization and intellectualization of agricultural technology management in precision farming.

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TRANSGENIC TOMATO PLANTS (*Solanum lycopersicum* L.): DIRECT METHODS OF GENE TRANSFER AND FACTORS AFFECTING TRANSFORMATION EFFICIENCY

(review)

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Abstract

Tomato (*Solanum lycopersicum* L.) is the most important food crop which is also widely used as a model plant in molecular genetic investigations of vegetative development and reproductive biology, plant resistance to abiotic and biotic stresses, plant-microbe association and symbiosis, etc., that have both basic and applied value. The production of transgenic tomato plants expressing foreign heterologous genes, as well as with induced silencing or knockout of their own genes, is an important part of modern plant physiology. There are two radically different approaches to introducing foreign DNA into the tomato genome. The first method is based on the natural mechanism of infection with plant-associated bacterial pathogen *Agrobacterium* sp. (*A. tumefaciens.* or *A. rhizogenes*), followed by T-DNA transfer and insertion into the plant genome (*Agrobacterim*-mediated transformation). The second approach is based on the direct introducing of foreign DNA into the plant cells through the plasma membrane by chemical (Ca^{2+}, polyethylene glycol, PEG) or physical exposure (electrical impulse or excessive pressure) (direct methods of tomato genetic transformation). Transgenic tomato plants can be produced both by the classical tissue culture-based transformation procedure and in planta transformation. This review article discusses classical direct methods for introducing foreign DNA into the tomato genome (chemical-mediated transfection, protoplast electroporation, microinjection, biolistic transformation), and in planta transformation methods (pollen-tube pathway, electroporation of mature seed embryo). The review considers features of producing tomato plants both with transient transgene expression and stably inherited insertion into the nuclear or plastid genomes are considered. In addition, the factors affecting the efficiency of transformation are analyzed in detail. A separate section is devoted to the direct tomato genetic transformation methods for delivering various genome editing tools (ZFNs, TALEN, CRISPR/Cas, base editing, prime editing) that have become widespread in the past five years.

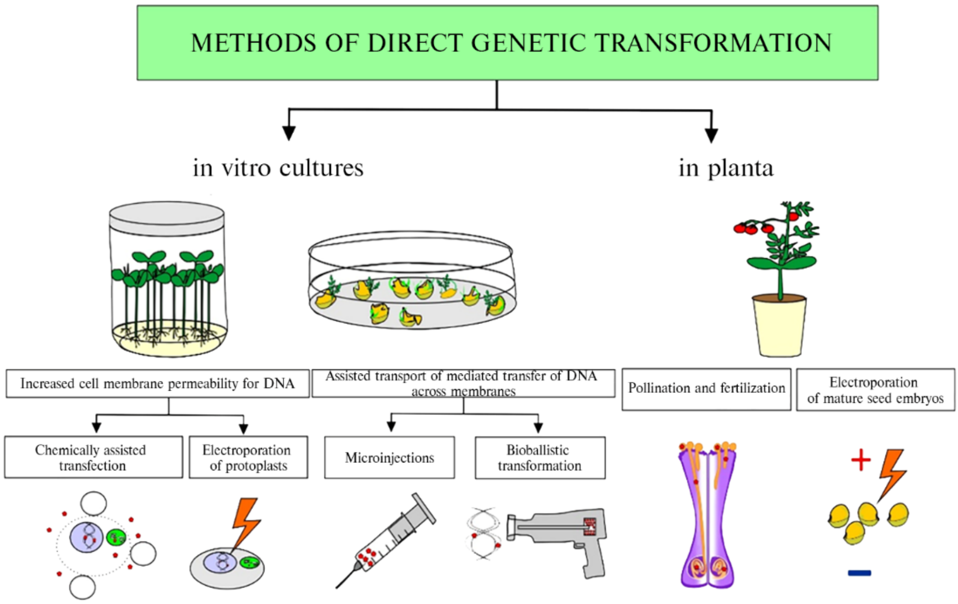
Keywords: *Solanum lycopersicum* L., electroporation, PEG-mediated transformation, microinjection, biolistic transformation, transformation in planta, genome editing

Tomato (*Solanum lycopersicum* L.) is important food crop, which ranks second after potatoes among agricultural vegetable plants in terms of the gross harvest of marketable products. Thus, according to the Food and Agriculture Organization of the United Nations (FAO), the global gross harvest of tomato fruits in 2020 amounted to approx. 186.8 million tons when grown on an area of 5.1 million hectares, of which Russia accounted for 2.9 million tons (approx. 1.6%) with an area of 80.7 thousand hectares [1]. In Russia, tomato is grown under various agroecological conditions, both in open and protected ground (about 60 and 40% of the gross harvest, respectively) [2, 3]. The main areas of cultivation of this crop are concentrated mainly in the southern regions of the country (Krasnodar and Stavropol Territories, as well as the Volga and Central Chernozem regions), the territories of which are more or less prone to primary and/or secondary salinization, as well as other edaphic stresses (4) . Thus, more than 31% of the soils in the Astrakhan region are characterized by a high degree of salinity (the concentration of Na^{+} and SO_4^{2-} ions reaches 7.1 and 12.5 mM per 100 g of soil, respectively), and about 20% of the soils are represented by solonetzic complexes [5]. In addition, the tomato has more than 45 infectious diseases of bacterial, fungal, viral and viroid etiology, which are distributed to varying degrees on the territory of the Russian Federation [6, 7]. As a result, increasing resistance to abiotic and biotic stress factors is one of the priority requirements for modern varieties and hybrids of tomato to realize potential yields. Therefore, it is necessary to constantly expand the range of crops using both traditional breeding approaches and modern methods of biotechnology and genetic engineering [8-10].

In addition to its great practical significance, the tomato is widely used as a model object in various fundamental studies affecting the issues of vegetative development and reproductive biology [11, 12], studying the mechanisms of plant resistance to abiotic and biotic stresses [9, 10, 13], and associative symbiosis with microorganisms [14, 15], meiotic recombination [16] and many others. The choice of such a model object is due to the fact that the tomato is a relatively unpretentious self-pollinating plant in cultivation with a short growing season, high reproductive potential, which is also capable of rapid vegetative reproduction and distant hybridization with some species of the genus *Solanum* [17]. The cultivated tomato and related wild species are diploids, the karyotype of which is represented by 24 chromosomes ($2n = 2\times12 = 24$). In addition, the tomato serves as a convenient model object due to the large number of morphological characters that are clearly identifiable at different stages of ontogeny [18, 19], as well as the availability of detailed genetic and molecular maps [20] (especially after the complete sequencing of the tomato genome in 2012) [21] and developed efficient and reproducible protocols for in vitro cultivation of isolated tissues [10, 22]. These factors ensured the widespread use of transgenic tomato plants as an experimental model for studying the role of expression of heterologous genes of various origins in fundamental biological processes.

Numerous accumulated data indicate that various genetic engineering strategies can significantly accelerate the creation of new tomato genotypes with traits that are difficult or impossible to achieve using traditional breeding. For example, it is possible to increase the resistance of tomato plants to phytopathogens by hybridizing tomato with related wild-growing species, such as *S. pimpinellifolium* L. and *S. habrochaites* S. Knapp & D.M. Spooner, followed by numerous backcrosses [23, 24]. However, only relatively closely related species capable of crossing with cultivated tomato can participate in such breeding programs; the process takes up to 15-20 years [25]. *S. lycopersicum* plants with increased resistance to fungal and bacterial pathogens can be obtained using various genetic engineering strategies: expression of heterologous genes of PR proteins (pathogenesis-related proteins synthesized in a plant cell during a pathogen attack) and antimicrobial peptides of plant, animal and fungal origin; metabolic engineering of phytoalexins and hormones; using resistance genes (R-genes); inhibition of toxic products of the pathogen; lignification of the plant cell wall; activation of plant defense reactions, etc.) [9]. The duration of the selection process is significantly reduced.

Both nuclear and plastid genome can be subjected to genetic transformation. The latter option has a number of undoubted advantages, since foreign genes integrated into the plastid genome are characterized by increased expression due to the characteristics of plastid DNA [26, 27] — a large number of plastome gene copies per cell (especially the level of transgene expression regardless of the insertion position in plastid DNA due to the lack of compact chromatin packing [30); the absence of epigenetic effects and the resulting silencing of transgenes [29, 30]. All these factors make it possible to increase the yield of transplastomic gene expression product to 40% of total soluble protein (TSP) [28, 29, 31]. Thus, transplastomic plants serve as promising bioreactors for the production of heterologous proteins for medical and veterinary purposes (32-34). Also, polycistronic cassettes are successfully expressed in plastids, which simplifies the co-transformation of one target by many different genes [29]. An undoubted ecological advantage of transgene localization in plastids is maternal inheritance of the plastome, which leads to the absence of the transgene in pollen and the impossibility of genetic contamination of the population [29, 33].



Classification of direct methods of tomato plants genetic transformation.

There are two fundamentally different approaches for introducing foreign DNA into the tomato genome (Fig.). The first one (method of agrobacterial or Agrobacterim-mediated transformation) is based on the natural mechanism of infection of plants with a bacterial pathogen of the genus *Agrobacterim* (*A. tumefaciens* or *A. rhizogenes*) and the transfer of foreign DNA into the plant genome mediated by it [35-37]. The second approach (see Fig.) is based on the direct delivery of foreign DNA into the plant cell through the plasmalemma using chemicals (Ca^{2+}, polyethylene glycol, PEG) or physical effects (electrical impulse or high pressure) (the so-called direct methods of tomato genetic transformation). In this case, transgenic plants can be obtained both by the classical method using the method of culture of isolated organs and tissues in vitro, and without it (transformation in planta) (see Fig.). Regardless of the method used to introduce foreign DNA into the tomato genome, the integration process is random [38].

It should be noted that each of the direct methods for introducing a transgene into the tomato genome has both advantages and disadvantages [39], however, all these methods are used much less frequently than agrobacterial transformation. The main reasons are their low efficiency (especially in the case of chemically mediated transfection, protoplast electroporation and microinjection), the need for specialized equipment ("gene gun", micromanipulator) and highly qualified personnel, as well as the high copy number of tandemly arranged insertions in plant DNA during bioballistic transformation, leading to low expression or silencing of the transgene [39-41]. However, in the last decade, in connection with the development of technologies for site-specific editing of the plant cell genome (including the tomato model) [42] with the participation of chimeric proteins and nucleoproteins created on the basis of bacterial or yeast endonucleases [43-46], methods of direct genetic transformations are being used more and more.

In addition, bioballistic and PEG-mediated transformation of protoplasts remain an essential tool for creating transplastomic tomato plants [34, 47].

In this review article, we systematized the available experimental data on the genetic transformation of tomato by the direct methods listed above, and analyzed various factors that determine the efficiency of the transformation process.

Chemically mediated transfection. The transformation of cells with chemicals that facilitate the transfer of DNA across the membrane was first carried out in the 1970s. In particular, the fundamental possibility of introducing labeled exogenous DNA into the protoplasts of *Ammi visnaga* (L.) Lam. [48] and *Nicotiana tabacum* (L.) [49] using various chemical compounds.

The calcium phosphate method was first used in 1973 for the genetic transformation of human cells with foreign DNA by F.L. Graham and A.J. Van der Eb [50]. The essence of the method is as follows: first, a $CaCl_2$ solution is added to a buffer solution containing DNA, as a result of which the negatively charged phosphate groups of DNA molecules bind to Ca^{2+} ions, and then a phosphate buffer is added to obtain a $Ca_3(PO_4)_2$ precipitate. The resulting DNA-containing solution is added to a suspension culture of isolated protoplasts devoid of a cell wall, which significantly hinders the penetration of macromolecules into the cell. In the first work on the transformation of tomato protoplasts by the described method, its maximum efficiency was 2% [51]. The transgenic nature of six callus aggregates was proved, however, later it was not possible to obtain full-fledged regenerated shoots from them. The authors demonstrated that the choice of plasmid type for genetic transformation has a decisive influence on the efficiency of obtaining transgenic callus tissues.

The use of PEG for DNA transfer across the plasmalemma was first tested for the genetic transformation of protoplasts of two types of tobacco (52). In addition to PEG, the buffer solution for DNA precipitation also contains $MgCl_2$. As a result of PEG-mediated transformation of tomato plastids with the pSSH1 plasmid, transplastomic plants resistant to the selective antibiotic spectinomycin were obtained. The transformation efficiency (TE) value calculated from restriction mapping was 1.5×10^{-6} [53]. S. Ray et al. [54] modified the method of chemically mediated transfection of the tomato plastid genome by a simultaneous presence of PEG and $CaCl_2$ in the buffer solution for DNA precipitation, as well as the addition of an osmotic agent, mannitol, to the solution. As a result, using the pCambia1302 plasmid, the selective and reporter genes (*nptII* and *gfp*, respectively) were integrated into plastid DNA. The authors found that PEG-4000 is more preferable than PEG-6000 due to the greater survival of protoplasts. In addition, a critical factor to maintain the viability of protoplasts was the duration of their incubation in a buffer solution after transformation and before being transferred to a nutrient medium to induce morphogenesis processes. The optimal value was 24 h, while longer incubation negatively affected the viability of protoplasts due to the toxic effect of PEG. The transgenic status of the resulting regenerants was confirmed by polymerase chain reaction

(PCR), as well as fluorescent analysis of the reporter gene expression; The authors did not define TE [54].

Microinjection. The method was developed for the delivery of nucleic acid macromolecules to human and animal cells [55, 56]. In this case, the plasmalemma of the cell is mechanically pierced with a microneedle - a very thin glass pipette with an outer diameter of 1-2 microns, which contains dissolved DNA. Transformation is performed by a specially trained operator under a microscope equipped with a micromanipulator.

The application of this method is significantly complicated by the presence of a strong cell wall and a large vacuole in a plant cell. The cell wall makes it difficult to visualize the nucleus, and its fragments clog the microneedle, so the microinjection method was developed for the transformation of naked protoplasts. Accuracy of microneedle targeting of specific cell compartments is improved by immobilizing protoplasts during microinjection, for example, if cells are placed on agar medium [57] or attached to a glass slide with poly-L-lysine (58). H. Morikawa and Y. Yamada [59] developed a mechanical method for holding protoplasts using additional pipettes; they also used fluorescent labels to visualize the DNA introduced into the cell [59]. The accuracy of micromanipulation is critical, since when the microneedle enters the vacuole, aliet DNA is destroyed by hydrolytic enzymes; in addition, disruption of the integrity of the vacuole can cause cell rupture and death due to the entry of toxic metabolites into the cytoplasm [60].

In the first works on the model of protoplasts isolated from hypocotyls of *Brassica napus* L., the survival of protoplasts after the introduction of alien DNA into them, as well as their subsequent ability to divide and form microcallus with a frequency of up to 70, 65 and 50%, respectively, was estimated. The authors found that the efficiency of these processes is affected by the following factors: genotype, age of the intact explant, the composition of the nutrient medium for cultivating protoplasts, and the pH of the buffer solution [61]. However, transgenic plants were subsequently obtained only for a limited number of crops, including plants of the *Solanaceae* family — petunias [62], tobacco [63], and barley [64]. The experimental work of Japanese researchers on the genetic transformation of intact tomato callus cells by microinjection was one of the first published in 1988. Using the pE2KX plasmid containing the *nptII* gene, which causes resistance to aminoglycoside antibiotics, the authors optimized the parameters that determine TE, the size of the microneedle (the use of a microneedle with an outer diameter of more than 0.3 μm leads to irreversible damage to cell structures and the death of 90-95% of the transformed cells) and the duration of the microinjection procedure. If it exceeds 20 s, irreversible structural and functional disorganization of the nucleus and other membrane organelles occurs). As a result of the selective selection of surviving injected cells on a nutrient medium with the addition of kanamycin, the frequency of callus aggregate formation after 1 month of cultivation was 22%; their transgenic nature was confirmed by PCR analysis for the presence of the *npt II* gene [65]. The TE values, which varied within 15-20% depending on the studied factors, were comparable with those obtained in alfalfa protoplast culture (15-26%) with the introduction of various plasmids (pTiC58, pMON8015, pMON120, pAL4404/pMON120) [66].

Electroporation. The essence of the method lies in the fact that under the influence of an electric pulse, pores with a diameter of about 30 nm are formed in the plasma membrane of the cell, which exist for several minutes, followed by the restoration of the normal state of the membrane. The short-term formation of pores is sufficient for the penetration of large water-soluble macromolecules, in particular DNA, into the cytoplasm of the cell [67-70]. Transformation is carried out with the help of an electroporator device, which consists of a cuvette, a pair of electrodes, and a pulse generator of a given shape [71]. A suspension of transformable cells and vector constructs with target genes are added to a cuvette with a buffer solution, after which an electrical pulse (usually rectangular or exponential) is passed through the solution [72]. The highest ET is achieved at a field strength of 1-20 kV/cm and a pulse duration of 1-30 ms, however, for each type of cell, the optimal values are selected empirically. The critical values of these physical parameters for the formation of pores and successful transport of DNA through the plasma membrane of a cell are determined by its own membrane potential, as well as by the potential arising under the action of an external electric field [73]. TE in most cases has a random component, since cells in solution are under different conditions and, therefore, acquire different potentials [74]. TE can be increased by adding various components to the electroporation buffer, such as Ca^{2+} or Mg^{2+} ions, PEG to increase membrane permeabilization [75], or 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) to maintain for the optimal pH value [76].

A significant limitation of the wide application of the electroporation method for plant objects is the presence of a cell wall [77, 78]. The use of a culture of protoplasts devoid of a cell wall significantly reduces ET, since the subsequent production of full-fledged regenerated plants from them is extremely difficult for many species. Obtaining stable transgenic lines of a number of cultures turned out to be possible in the case of adapting the electroporation technique developed for isolated protoplasts for use on intact cells. This approach proved to be effective in obtaining a stably inherited transgene insertion in the maize genome; however, to achieve a positive result, the authors demonstrated the need for partial disruption of the cell wall and increased membrane permeabilization in transformed cells [79].

The production of transgenic tomato plants by electroporation was first reported back in 1989 by two independent groups of researchers [80, 81]. So, C. Bellini et al. [80] introduced alien DNA into the protoplasts of two species of the genus *Solanum* — *S. lycopersicum* L. and wild-growing tomato species *S. peruvianum* L. TE calculated as the ratio of the number of transformed colonies resistant to a selective antibiotic to their total number, varied within 0.3-2.5%. The maximum TE value was achieved when protoplasts were treated with a three-time pulse of 100 μs duration, creating a voltage of 250 V or 300 V. The regenerated plants were successfully obtained from transformed protoplasts [80]. M. Tsukada et al. [81) used single exponential pulses resulting from the rapid discharge of a pre-charged capacitor for the genetic transformation of protoplasts of cultivated tomato cv. Petit Tomato. An electric field strength of 1 kV/cm with a capacitor discharge of 47 μF provided the best results [81].

On the model of protoplasts of the wild species *S. peruvianum* L., we optimized a number of physical and physiological factors affecting the process of introducing foreign DNA, such as the composition of the buffer solution for electroporation (Ca^{2+}- or Mg^{2+}-containing buffer solution with or without PEG), cell concentration in suspension of protoplasts, their pretreatment (heat shock), as well as pulse parameters [75]. The maximum efficiency of the electroporation process was achieved under the following conditions: the use of a Ca^{2+} buffer solution and a heat shock for 5 min at a temperature of 45 °C, followed by the addition of 8% PEG, as well as a rectangular pulse with a duration of 30 μs and an electric field strength of 1.5 kV/cm.

1. Bioballistic method of tomato genetic transformation and factors affecting its efficacy

Transformation method[1] and transgene localization[2]	Explant, genotype	Device (manufacturer)	Parameters of bioballistic transformation			Selective and/or reporter gene[4]	TE, %	Note	References
			distance between the macrocarrier and stop screen, cm	helium pressure, psi	type[3] and microparticle size, μm				
T	Suspension cell culture of tomato variety VFNT Cherry and *Solanum pennellii* Correll.	PDS-1000/He™ (Bio-Rad)	8.5	nd	W, 1.0	*nptII*, *uidA* (*gus*)	1.2 (VFNT Cherry) and 45.0	TE was the ratio of the number of kanamycin-resistant calli to the number of cells in which transient expression of the reporter gene occurred after 48-h shell transformation	[83]
T	Leaves, 5-8 mm unripe fruits of tomato variety VFNT Cherry LA 1221	PDS-1000/He™ (Bio-Rad)	8.5	1500, 1800 (optimum 1800)	Au, 0.4-1.2	*luc или uidA* (*gus)*	nd	Optimyzed bioballistic transformation parameters for transient expression	[89]
SP	Leaves of tomato variety IAC-Santa Clara	PDS-1000/He™ (DuPont)	nd	1100	Au, 0.6	*aadA*	2.5* 1.15**	* The ratio of the number of calli resistant to spectinomycin to the total number of transformed explants ** The ratio of the number of regenerants with PCR-confirmed transgene insertion to the initial number of transformed explants	[28]
SP	Leaves of tomato line IPA-6	PDS-1000/He™ (Bio-Rad) с Hepta Adaptor (Mologen)	nd	nd	Au, 0.6	*aadA*	nd	Production of stably inherited transplastomic plants expressing bacterial lycopene-β-cyclase with increased herbicide resistance and a 4-fold increase in provitamin A content in fruits	[90]
SN	Parts of hypocotyl of tomato variety CastleRock	PDS-1000/He™ (Bio-Rad)	6.0 and 9.0	1350	Au, nd	*bar*, *uidA* (*gus)*	26.5	TE was the ratio of the number of primary transformants with PCR-confirmed status to the total number of regenerants	[91]

Continued Table 1

T	Apical meristem, cotyledons and hypocotyl parts of tomato line IPA-3	PDS-1000/He™ (Bio-Rad)	2.5-10.0 (optimum 7.5)	1100	W, 0.6	*uidA* (*gus)*	from 22.69 to 36.56	TE was the ratio of the number of explants with GUS activity to the total number of transformed explants; the maximum TE value (36.56%) when using parts of hypocotyls	]84]
SN	Parts of hypocotyl of tomato variety CastleRock	PDS-1000/He™ (Bio-Rad)	6.0 and 9.0	1350	Au, 1.0	*hpt*	42.5	ET was the ratio of the number of transgenic regenerants confirmed by PCR analysis to the total number of hygromycin-resistant regenerants	]92]
SP	Parts of hypocotyl with cotyledons (hypocotyledonary) of tomato variety CastleRock	PDS-1000/He™ (Bio-Rad)	6.0 and 9.0	1350	Au, 1.0	*hpt*	52.3	The same as in [92]	]93]
T, SP	Parts of leaves and fruits of tomato variety Ferum	Gene Gun Helios System (Bio-Rad)	nd	210	W, 1.1	*luc* или *uidA* (*gus)*	nd	Study of the activity of the fruit-specific promoter of the *SlPPC2* gene encoding tomato phosphoenolpyruvate carboxylase (EC 4.1.1.31)	]85]
T	Fragments of various parts of fruits of tomato line F-144	Scientz GJ-1000 (Ningbo Scientz Biotechnology Co., Ltd.)	1.0. 3.0 and 6.0 (optimum 6.0)	500, 650 and 1100 optimum 1100)[5]	W, 1.0	*uidA* (*gus)*	nd	The largest number of blue dots in GUS reaction (2456.91/cm^2) at the points where the tomato fruit is attached to peduncle	]86]
SP	Callus tissue derived from leaves of tomato variety Pusa Ruby	PDS-1000/He™ (Bio-Rad)	6.0. 9.0 and 12.0 (optimum 9.0)	1100	nd	*nptII*, *uidA* (*gus*)	75.0	The 9.0 cm distance between the macrocarrier and the stop screen provided the maximum TE value (75%)	]102]
T	Callus tissue derived from mature embryos of tomato variety Ventura	Salyaev's pneumatic gene gun [102]	nd	nd	W, 1.1-1.2	*uidA* (*gus*)	80.0	Transient expression of the *gus* gene was confirmed by histochemical method with X-Gluc[5]	]87]
SP	Callus tissue derived from mature embryos of tomato variety Ventura	Salyaev's pneumatic gene gun [102]	nd	nd	W, 1.1-1.2	nd	nd	Production of transplastomic tomato plants containing the *hpv16 L1* gene encoding the synthesis of the main antigenic protein of the highly oncogenic human papillomavirus type HPV16 L1 which is up to 5300 ng/mg of total soluble protein	]88]

Continued Table 1

SN	Parts of hypocotyl of tomato-variety Rutgers	PDS-1000/He™ (DuPont)	6.0 and 9.0 (optimum 9.0)	1200	Au, 0.73	*nptII*, *uidA* (*gus*)	nd	PCR analysis and the reporter gene expression, confirmed by histochemical staining for β-glucuronidase activity, indicated the production of transgenic plants	[94]
SP	Leveas of tomato variety Micro-tom	PDS-1000/He™ (Bio-Rad)	12.0	1100	Au, 0.6	*aadA*	nd	Production of stable transplastomic plants with induced RNA interference to control insect pests	[95]

N o t e. [1] — stable transformation (S) or transient expression (T); [2] — nuclear (N) or plastid (P) transformation; [3] — microparticles of gold (Au) or tungsten (W); [4] — *nptII* and *hpt*, selective genes for neomycin phosphotransferase II and hygromycin phosphotransferase of *Escherichia coli*, respectively; *aadA*, a selective gene encoding the enzyme aminoglycoside-3'-adenyltransferase; *uidA* (*gus*) and *luc*, reporter genes for β-glucuronidase and luciferase, respectively; [5] — X-Gluc (5-bromo-4-chloro-3-indolyl-β-D-glucuronide); excess pressure may be created not by helium but by nitrogen in the "gene gun" model; nd — no data.

Bioballistic transformation. This direct method for delivering nucleic acid macromolecules to plant cells was developed by John Sanford at Cornell University (USA) in 1984 [82]. The essence of the method is that DNA molecules are deposited on the surface of carriers — gold or tungsten microparticles [83-95] (Table 1) or nanoparticles [96], which are accelerated to a 300-600 m/s due to the helium pressure. They carry out the bombardment of various explants placed on the target. Since particles with such a high velocity successfully penetrate the cell wall, the bioballistic method is suitable for the transformation of intact cells, which has an important advantage in avoiding the steps of protoplast isolation and subsequent low-efficiency morphogenesis [83].

The development of a device for shelling microparticles (the so-called gene gun) began in 1984, and the idea was subsequently patented by the developers [97]. Subsequently, the original model was developed in modifications: He Biolistics Particle Delivery System (PDS-1000/Hetm; licensed by DuPont, USA); noncommercial variant of Accel™ Particle Gun (98); Particle Inflow Gun [99]; microtargeting device designed for transformation of apical meristems [100] and Helios Gene Gun (Bio-Rad, USA). The latter option does not require a vacuum chamber to hold the target tissue and can be used as a portable device [101].

Bioballistic transformation is the most widely used direct method for introducing foreign DNA into tomato cells, which is used both to study the transient expression of heterologous genes [83-87, 99] and to obtain transgenic plants with a stably inherited nuclear [85, 91-94, 102] or plastid [28, 88, 90, 95] transgene insertion (see Table 1). Most often, the shelling of tomato plant tissues with microparticles is carried out using the PDS-1000/He™ device from DuPont or Bio-Rad [28, 83, 84, 89-93, 94, 95] (see Table 1), significantly less commonly, serial devices from other manufacturers [86] or devices developed independently [103]. The target with transformable isolated cells or explants is installed in a vacuum chamber with a pressure of about 0.1 atm. At the moment of pressure release, the particles are ejected from the gun towards the target. Typically, cells in the center of the target die due to physical damage [104]. The applied model of the installation determines the following set of physical parameters of bioballistic transformation that affect the efficiency of the process: DNA concentration, type of microparticles and their size, varying from 0.4 to 1.2 μm, distance between the particle macrocarrier and the stopping screen (from 6.0 to 9 .0 mm), helium pressure (from 200 to 1800 psi), as well as the multiplicity of shots (single or multiple). These parameters are optimized taking into account the age and physiological characteristics of specific explants, the cells of which are subjected to shelling. Tomato transformation protocols have been developed to obtain stable and transient expression of heterologous genes on the model of suspension [83] and callus [87, 88, 102] cell cultures, apical meristem [84], hypocotyl fragments [84, 91, 92, 94], cotyledons [84], leaves [85, 89, 90, 95] and fruits of various degrees of maturity [85, 86, 89]. ET varies widely (from 1.2 to 80.0%) depending on the type of transformation (stable or transient), genotype characteristics, explant type, and many other physiological and physical factors (see Table 1). It is also important to note the lack of a universal methodology for determining ET, as a result of which the authors propose radically different methods for calculating this indicator (see note in Table 1), the values of which can differ even by an order of magnitude.

In 1995, stable transgenic plants of the tomato and its wild relative *S. pennellii* Correll were obtained for the first time by bioballistic transformation [83]. Suspension culture cells were subjected to shelling, which were subsequently cultivated on a nutrient medium to induce morphogenesis processes with the addition of a selective agent, kanamycin. The study used a yeast chromosome (YAC) vector and three types of plasmids carrying the *uidA* (*gus*) and *nptII* genes. The authors demonstrated transient expression of GUS in all cases, however, the number of selected kanamycin-resistant calli in the wild species was significantly greater than in the cultivar at a comparable level of transient expression. This fact indicates that stable transformation is genotype-specific, and the reason for this is the genetic determination of various genotypes and somatic tissues to in vitro morphogenesis [10, 22, 105, 106]. In general, the genotypes of representatives of the genus Solanum can be arranged in the following order according to their morphogenetic potential: closely related wild tomato species *S. pimpinellifolium* L., *S. peruvianum* L., and *S. glandulosum* (L.) Morong > *S. lycopersicum* L. [107); model genotypes with no practical value > commercially important F_1 varieties and hybrids [10]; cultivars *S. lycopersicum* L. > hybrids F_1 *S. lycopersicum* L. [108].

D. Ruma et al. [84] performed optimization of the physical parameters of bioballistic transformation during transient expression of the reporter gene uidA (gus) in various tomato explants of the IPA-3 line. The maximum ET (34.12, 36.56 and 22.69%, respectively, for the apical meristem, fragments of hypocotyls and cotyledons) was achieved by double bombardment of the explant with microparticles from a distance of 7.5 cm and a helium pressure of 1100 psi [84]. In addition, the authors studied the biological factors that affect the frequency of transient expression of the reporter gene. For one shot, the optimal amount of plasmid DNA was 1.89 μg per 1125 μg of microparticles. The use of DNA in excessive concentrations reduces ET due to the adhesion of microparticles [109]. Explant preculture reduced ET due to the loss of mechanical strength of cell walls in competent cells. Similar results were observed in the case of pretreatment of tomato explants with 0.3 M mannitol solution [84]. In another study, on the contrary, pretreatment of tomato leaves and fruits with osmotic (12% mannitol) before shelling led to a 30-fold increase in the level of expression of the heterologous luciferase gene [89]. L. Sun et al. [86) performed shelling with tungsten particles of tomato fruits of different maturity with the "gene gun" (Scientz GJ-1000, Scientz, China). The excess pressure was generated not with helium, but with nitrogen. The maximum TE was achieved with a single shelling of explants with microparticles (0.83 μg DNA per shot) from a distance of 6 cm at a nitrogen pressure of 1100 psi [86].

In order to obtain transgenic tomato plants with a stably inherited gene with a high frequency (26.5%), G.A. Abu-El-Heba et al. [91] proposed other parameters of bioballistic transformation, i.e., 1 μg aliqute of DNA, double shelling with gold microparticles at a 6 and 9 cm distance between the macrocarrier and the stop screen for the first and second shots, respectively; helium pressure of 1350 psi [91]. These values have been successfully applied in a number of research works for shelling fragments of hypocotyls, as well as other types of tomato plant tissue [92, 93, 102], taking into account the individual characteristics of the composition of the nutrient medium for the induction of morphogenesis.

Bioballistic transformation is the main method for creating transplastomic tomato plants, which were first obtained at the beginning of the 21st century based on the commercial variety IAC-Santa Clara [28]. The peculiarity of such plants is the inheritance of the transgene in generations along the maternal line. The selection of homoplastomic cells and plants is carried out on a selective nutrient medium with the addition of spectinomycin at concentrations of 300-500 mg/l [28, 90, 95] with a photoperiod of 16/8 h (day/night) and reduced illumination (15 or 25 μE) [28, 95]. Expression of the target gene was observed in different types of plastids: leaf chloroplasts, fruit and flower chromoplasts [28].

T r a n s f o r m a t i o n in planta. Transformation in planta makes it possible to obtain transgenic plants, bypassing the long and laborious stage of cultivating isolated organs and tissues in vitro. The main advantages of in planta transformation are the relative simplicity and speed, since these methods do not involve equipping the laboratory with specialized biotechnological equipment due to the absence of an in vitro culture stage, thereby eliminating somaclonal variability [110, 111]. Pollen can be considered the best material for in planta transformation: it can be obtained in large quantities, it contains haploid cells, and practically any of the methods previously tested for the transformation of protoplasts and intact cells can be used for transformation - electroporation, microinjection, DNA packaging into liposomes, bioballistic method, as well as *Agrobacterium*-mediated transformation [112].

During the germination of transformed pollen in planta, exogenous DNA enters the embryo sac, followed by the possible formation of a transgenic diploid embryo [113, 114]. This method, called the pollen tube pathway, was developed and first applied in 1974 on plants of the *Solanaceae* family *Petunia hybrida* Vilm. [115, 116] and *Nicotiana glauca* Graham [115], and also on barley plants [117]. The pollen tube method was effective to produce transgenic plants of cotton [118], barley [119], rye [120] and other crops, including various tomato genotypes [121-126].

The first work showing the possibility of genetic transformation of tomato using the pollen tube method was published in 1989 [121]. This study was continued by other works, in particular, transgenic hybrids of *S. lycopersicum* L. cv. Fakel and *Solanum penelii* Cor. [125]. In this case, the genetic transformation of pollen and the process of pollination were carried out simultaneously. Freshly dried pollen was placed in a nutrient medium supplemented with 15% sucrose, 0.018% boric acid, and 0.04% $Ca(NO_3)_2$, to which a solution of plasmid DNA was then added. Immediately after the addition of DNA, pollination was carried out with the transformed pollen of previously castrated and isolated flowers. ET calculated as the ratio of the number of kanamycin-resistant seedlings derived from immature embryos to the total number of transformed embryos was 2.2%. The ratio of the number of kanamycin-resistant and kanamycin-sensitive seedlings obtained from seeds was 3:1, which proves their transgenic status and indicates a single-locus model of transgene inheritance. The relatively low efficiency of transformation by the pollen tube pathway method is largely associated with the nuclease activity of germinating pollen, which degrades most of the exogenous DNA [112].

Another modification of the pollen tube method, based on an earlier work by N.V. Turbine et al. [117], involves the introduction of a DNA-containing solution into the ovule of an already fertilized flower. After pollination, the stigma is removed and a solution containing the target DNA sequence is injected with a fine needle [114]. This method has also been used to obtain transgenic tomato plants [122-124]. R. Wang et al. [124] studied the effect of genotype, plasmid DNA concentration, and components of the buffer solution for injection on the ET of tomato varieties Zhongshu 6, Liaoyuanduoli, and Jinguan 9 using a vector construct containing the selective *bar* gene, which causes resistance to phosphinothricin, as well as the yellow fluorescent reporter protein gene *yfp*. The authors established the absence of significant differences in ET between the studied varieties. At the same time, the highest yield of transgenic plants was provided by the addition of 600 ng/μl of plasmid DNA, as well as 5% sucrose, and 0.05% Silvet-L-77 surfactant to the solution for injection [124]. For the described modification of the pollen tube pathway method, the time interval from pollination to transformation and the preservation of the ovule upon removal of the pistil are critically important [123, 127]. It has been established that the introduction of foreign DNA should be carried out 24 hours after pollination; shortening this period reduces ET

[123]. As regards the safety of the ovule, various methods are used to break the integrity of the pistil before the introduction of alien DNA, for example, the complete removal of the pistil before injection [123] or the removal of only part of it (stigma with part of the style) [122]. The contribution of the latter factor is difficult to assess due to the low efficiency of the method as a whole. Depending on the above factors, ET ranges from 0.2% [124] to 1.4% [128].

A variation of the pollen tube pathway method is the agroinfiltration of foreign DNA into various plant generative organs [129, 130]. The effectiveness of various modifications of this method reaches an average of 3-4%. The procedure is actively used to obtain transgenic *Arabidopsis* plants and other cultures of the *Brassicaceae* family [131]. This method can be considered as a hybrid between pollen tube pathway and agrobacterial transformation. In tomato, the discussed method was successfully applied to introduce the *LFY* and *GUS* genes [132].

A highly efficient protocol for electroporation of mature tomato seeds was proposed by Z. Hilioti et al. [133] for the delivery of genome editing systems. The sterilized seeds were incubated for 12 h in the dark at a low temperature (10 °C) in a solution supplemented with 5% sucrose, 3% H_3BO_3, and 1.3 mM $Ca(NO_3)_2$. The swollen seeds were subjected to vacuumnation in a buffer solution (80 mM KCl, 5 mM $CaCl_2$, 10 mM HEPES, and 0.5 M mannitol) and kept on ice for 1 h. Electroporation of pretreated seeds was carried out in a buffer solution with the addition of plasmid DNA (50 μg per 200 μl of buffer) in the mode of three pulses of 4 ms each at a field strength of 6.25 kV/cm. The authors demonstrated that 65% of tomato plants grown from transfected seeds contained various mutations of the target gene, which indicates successful transformation of the embryos with the vector encoding ZFN nucleases and their subsequent expression in electroporated embryos [133].

Use of direct methods of tomato genetic transformation for the delivery of genome editing systems. Over the past 5 years, there has been a boom in research work devoted to the targeted introduction of changes in the tomato genome using genomic editing systems ZFNs (zinc-finger nucleases), TALEN (transcription activator-like effector nucleases) and CRISPR/Cas (clustered regularly interspaced short palindromic repeats) [43, 134]. Depending on the system used, various variants of genomic editing can be carried out - through knockout mutations resulting from insertions or deletions of a part of nucleotides in the editing site due to non-homologous combination of repaired ends or through homologous recombination; knock-in gene editing by introducing exogenous oligonucleotides or longer DNA fragments after the introduction of breaks and subsequent homologous recombination; the introduction of single nucleotide substitutions due to the deamination of nitrogenous bases (CBE, cytosine base editors, ABE, adenine base editors). The mechanisms of action of the genomic editing systems ZFNs, TALEN, CRISPR/Cas, CBE, and ABE are considered in detail in a number of review articles [135-139].

To date, various methods of editing the tomato genome are widely used in both fundamental [140] and pronounced applied studies, for example, in order to increase plant resistance to abiotic [141, 142] and biotic [143-146] stressors of various nature, increase keeping quality of fruits and improving their quality [147-152], as well as to accelerate the process of domestication of wild *Solanum* species [153, 154]. Delivery of genome editing systems is carried out mainly through agrobacterial transformation with vector constructs expressing these systems. Direct methods for introducing alien DNA to obtain genome-edited tomato plants also occupy a significant place in this list (Table 2).

2. Direct methods for delivering genome editing systems to tomato cells

Editing system	Delivery method	Genotype	Gene	Trait	Editing efficacy, %	References
TALEN	PEG-Ca^{2+}-mediated transformation	Micro-Tom	*ANT1*	Anthocyanin biosynthesis; purple color	7.28	[155]
CRISPR/Cas9	PEG-Ca^{2+}-mediated transformation	Micro-Tom	*ANT1*	Anthocyanin biosynthesis; фиолетовой окраски	2.75-8.8	[155]
	PEG-mediated transformation	Micro-Tom	*CCD7* *CCD8*	Strigolactone biosynthesis	30 90	[156]
	PEG-Ca^{2+}-mediated transformation	*Solanum peruvianum* L.	*SpRDR6* *SpSGS3* *SpPR-1* *SpMlo1* *SpProSys*	Resistance to plant pfthogenes	13.2 8.3 13.9 63.6 45.8	[157]
ZFNs	Electroporation of mature seed embryos	Heinz 1706	*LIL4*	Transcription factor to control the development of cotyledon, true leaves, flowering and fruit ripening	65	[133]
Prime editing	Биобаллистика	Micro-Tom	*NanoLucM*	Back mutation that restores NanoLuc luciferase activity	0.26[a]	[158]

N o t e. [a] — transient expression efficacy.

Thus, using the TALEN and CRISPR/Cas9 systems and PEG-Ca^{2+}-mediated transformation of tomato protoplasts of the Micro-Tom model variety, the CaMV35S promoter was accurately inserted between the promoter and transcribed regions of the *ANT1* gene, which controls anthocyanin biosynthesis. The constitutive promoter-mediated overexpression of the *ANT1* gene contributed to the ectopic accumulation of the pigment in plant tissues. Depending on the type of genetic construct and genomic editing system, ET varied from 2.75 to 8.8%. More than two thirds of the transgene insertions were accurate and stably inherited in the T_1 seed generation according to Mendelian segregation [155].

The PEG-mediated delivery of the CRISPR/Cas9 system into tomato protoplasts made it possible to edit two genes for carotenoid-cleaving dioxygenases (*CCD7* and *CCD8*) involved in strigolactone biosynthesis. In this case, multiplexing was used, that is, the simultaneous targeting of several guide RNAs to both genes. For transfection of protoplasts, the authors used a multicomponent buffer solution containing 12.5% PEG-4000. As a result, out of 50 randomly selected callus aggregates formed in the protoplast culture, one (2%) and five (10%) had monoallelic mutations (in the *CCD7* and *CCD8* genes, respectively), while 13 (26%) and 36 (72%)) calluses contained biallelic mutations of the same genes (homozygous or heterozygous state) [156].

Y.C. Lin et al. [157] obtained independent regenerants with point mutations introduced by CRISPR/Cas9 into the following genes, the expression products of which confer resistance to phytopathogens, from the protoplast culture of *S. peruvianum*: *SpRDR6* (RNA-dependent RNA polymerase 6), *SpSGS3* (suppressor gene silencing 3) (two key RNA silencing genes mediating protection against tomato yellow leaf curl virus), *SpPR-1* (pathogenesis-related protein-1), *SpProSys* (prosystemin), and *SpMlo1* (one of the gene family of the O locus causing resistance to snow mold). As a result, the frequency of editing, depending on the gene, varied between 8.3 to 63.6% [157].

The use of mature embryos of tomato cv. Heinz 1706 seeds as explants for electroporation with plasmids with the ZFN sequence made it possible to obtain independent lines with the edited *LIL4* gene encoding the transcription factor LEAFY COTYLEDON1-LIKE4, which controls the development of cotyledon and true leaves, as well as flowering and fruiting. It was found that 65% of tomato plants grown from transfected seeds contained various mutations of the target gene

[133].

The delivery of genomic editing systems is also carried out using the bioballistic method. An example is the editing of the Micro-Tom tomato genome using the prime editing system based on the Cas9 nuclease, cross-linked with mouse leukemia virus (MuLV) reverse transcriptase and containing a new guide RNA variant, prime editing guide RNA (pegRNA), which not only directs the nuclease to the desired site of DNA, but also serves as a template encoding changes [158]. The efficiency of genome editing using this technology was 0.26% (the indicator was estimated by means of restored transient expression of luciferase 7 days after microparticle bombardment).

Thus, this review article discusses various direct methods (chemically mediated transformation, microinjection, electroporation, bioballistic transformation) of introducing foreign DNA to obtain transgenic tomato plants, as well as factors of various nature (physical, genetic, and physiological) that affect the efficiency of this process. The first three methods are characterized by low efficiency, and therefore have not been widely used. The most common direct method of tomato genetic transformation is the bioballistic method, which produces plants with a stably inherited transgene insertion in both the nuclear and plastid genomes with varying efficiency. It also should be noted that a direct comparison of transformation efficiency (TE) is not always possible, since different authors use different methods for its determination. In addition, in a number of research papers, no TE occurred, since, apparently, the resulting transgenic tomato plants were single or TE determination was not a goal. It should be noted that with the wide development of various genome editing systems, direct methods of tomato genetic transformation are used more and more often, especially with protoplast culture.

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RANGE EXPANSION AND INCREASING DAMAGE POTENTIAL OF PHYTOPHAGOUS SHIELD BUGS (Heteroptera: Pentatomidae)

(review)

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Abstract

During a few recent decades, in many regions of the world, there have been recorded expansion of ranges and an increase in the harmfulness of many stink bug species (Heteroptera: Pentatomidae) (A.R. Panizzi, 2015; J.E. McPherson, 2018). A leading role in these processes is probably played by the current climate change and unintentional introduction of phytophagous pentatomids as a result of intensified transportation of goods and development of tourism, coupling with natural polyphagy and high migratory potential of many pentatomids (D.L. Musolin, A.Kh. Saulich, 2012, A.M. Wallner et al., 2014; T. Haye et al., 2015; T.C. Leskey, A.L. Nielsen, 2018). In the south of Russia, since early XXI century there have been numerous records of increased population density and substantial damage caused to soybean, vegetable, fruit, and berry crops caused by the southern green stink bug *Nezara viridula* (L.) that previously had only limited distribution and damage in the region (M.V. Pushnya et al., 2017; A.S. Zamotailov et al., 2018). In Krasnodar Krai and Republics of Adygea and Crimea, losses of tomatoes, beans, cabbage, grapes, raspberries and other crops caused by this pest reached 70—90 % in some places in 2017—2019. On the Black Sea coast of the Caucasus (in Russia, Abkhazia, and Georgia), agricultural and ornamental crops are currently also seriously damaged by the invasive brown marmorated stink bug *Halyomorpha halys* (Stel) that was introduced to the region less than 10 years ago (I.M. Mityushev, 2016; D.L. Musolin et al., 2018). In different parts of its invasive range, this polyphagous pentatomid demonstrates tendencies to expand its' host plant range (D. Lupi et al., 2017; M.-A. Aghaee et al., 2018; S. Francati et al., 2021; V. Zakharchenko et al., 2020). At the same time, various wild plant species growing along forest edges and forest belts have recently become the major reserves of *N. viridula* and *H. halys* in the Caucasus and this greatly complicates the control of these pests (B.A. Borisov et al., 2020). Studies of seasonal development of the native Italian striped bug *Graphosoma lineatum* (L.) in the forest-steppe zone of the Belgorod Province demonstrated that currently this species often produces two annual generations, whereas in the 1990s the species had two generations during the growing season only in the exceptionally warm years with temperatures above the mean level (D.L. Musolin, A.Kh. Saulich, 2001). Currently, in European countries and Russia, a number of pentatomids, e.g., *Palomena prasina* (L.), *Dolycoris baccarum* (L.), *Eurydema ornata* (L.), *Pentatoma rufipes* (L.), and *Rhaphigaster nebulosa* (Poda), have increased population densities what is accompanied by increased damage caused by these species to cultivated crops and wild plants. In Central America, *Antiteuchus innocens* Engleman et Rolston was not previously considered a serious pest, but in recent years an increased abundance of this pentatomid has been recorded in Mexico, what lead to a weakening of pine forests (F. Holguнн-Melйndez et al., 2019). High population densities and increased damage to crop production caused by stink bugs are also facilitated by the absence or slow development of control measures against these invasive pests.

Key words: Hemiptera, Heteroptera, Pentatomidae, phytophagous insects, pests, harmfulness, population density dynamics, invasive species, climate change, *Nezara viridula*, *Halyomorpha halys*

Stink bugs (Heteroptera: Pentatomidae) are the largest family of hemipterans, including 10 subfamilies, 940 genera and approx. 4 950 species of phytophages, less often predators or zoophytophages [1-6]. The majority of pentatomid species are characterized by an annual life cycle synchronized with seasonal cycles of development of host plants, and overwintering at the adult stage [7]. In the Russian entomological literature of the XX century, pentatomids appeared mainly as secondary pests of almost all groups of agricultural crops [8-9]: cereals [10], technical [11], vegetables and potatoes [12], annual and perennial grasses [13], fruit and berry crops [14], sugar beet [15].

According to the most complete reference book among agricultural pests [16], only 10-15 species of the pentatomids were periodically economically significant. These are several species in the genus *Eurydema* (*E. oleracea* (L.), *E. ventralis* Kolenati, *E. ornata* (L.), *E. maracandica* Osh. et al.), trophically related to the species of the family *Brassicaceae*; bugs of the genus *Aelia* (*A. acuminata* (L.), *A. klugii* Hahn, *A. rostrata* Bohemian, *A. sibirica* Reuter) on cereals; *Graphosoma lineatum* (L.), harmful to plants of the family *Apiaceae*; polyphages *Carpocoris purpureipennis* (De Geer) and *Dolycoris baccarum* (L.). Nevertheless, according to the economic damage extent, all of them were much inferior to bugs from a closely related family of shield bugs (Scutelleridae) — *Eurygaster integriceps* Puton, *E. austriaca* (Schrank) and *E. maura* (L.) [17].

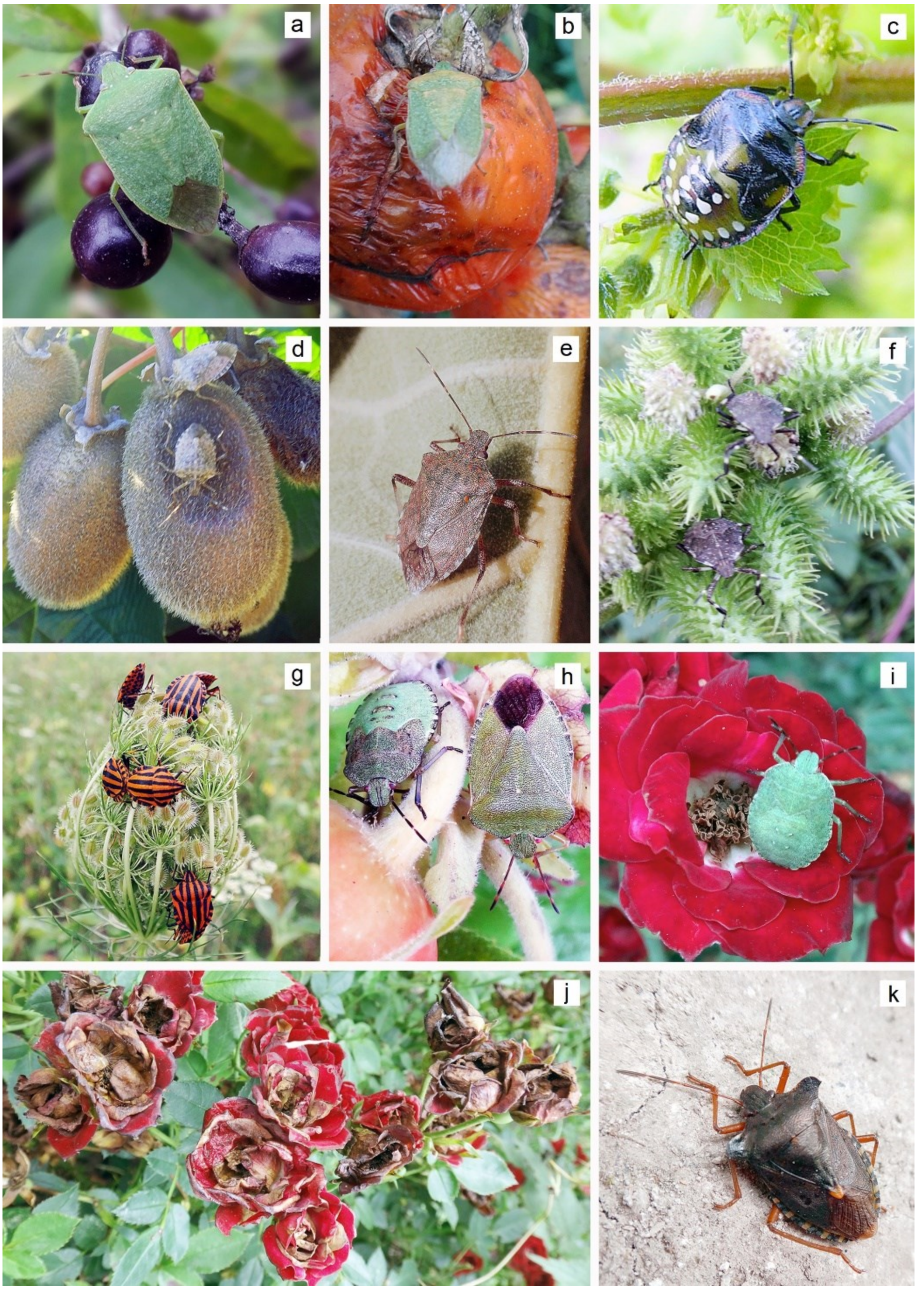
Foreign researchers also pointed to the relatively low harmfulness of pentatomids in the past [18, 19]. The most dangerous species in various regions previously included *Murgantia histrionica* (Hahn) (feeding on cruciferous crops) [20]; *Mormidea quinqueluteum* (Lichtenstein) and *Arvelius albopunctatus* (De Geer) (on nightshades and rice, respectively) [21, 22]; *Aelia furcula* Fieb. (on wheat *Triticum* L. and barley *Hordeum vulgare* L.) [23]; *Agonoscelis pubescens* (Thunberg) [on sorghum *Sorghum* Moench and sesame *Sesamum indicum* L.) [24].

However, since the end of the XX century, many insect species, including pentatomids, have had a noticeable expansion of their ranges [18, 25-28], an increase in population density [29-31], and/or in harmfulness [30-32] in different parts of their ranges. This is probably due to global climate change (which usually manifests itself in milder winter conditions, lengthening of the growing season, etc.) [26, 27, 33-36], intensification of global transport flows and increase in mass tourism [35, 37].

The purpose of this review is to comprehensively identify and analyze the causes and scale of numerous, but disparate examples of changes in the ranges and the level of harmfulness of pentatomids in Russia and other countries in early XXI century.

The southern green stink bug *Nezara viridula* L. (Fig., a-c), originating from northern Africa [38], in the later XX century, has begun to spread around the world as one of the first among pentatomids, causing economic damage to various agricultural crops, especially soybeans (*Glycine max* (L.) Merr.) [39]. During the recent decades, the situation with this phytophagous pest has worsened in many European countries: Great Britain [40], Romania [41], Germany [42], Slovakia [43], Poland [44], the Czech Republic [45], as well as in Turkey [46], Cuba [47], in Argentina [48], Brazil [49], India [50], Japan [51, 52], Australia [53], etc.

The harmfulness of this species is greatly impaired by its ability to transmit phytopathogenic microorganisms [54-56]. As it was shown, the shift of the northern border of its range is directly related to the increase in winter temperatures [57].



Pentatomids on the plants they damage. The southern green stink bug *Nezara viridula*: a — an adult on fruits of common privet (*Ligustrum vulgare*) (Republic of Adygea, 2019), b — an adult on a fruit of tomato (*Solanum lycopersicum*) (Republic of Adygea, 2018), c — a nymph on a leaf of shrub perilla (*Perilla frutescens* (L.) Britton) (Sochi, 2021); brown marmorated stink bug *Halyomorpha halys* (Sochi, 2018): d — an adult on fruits of kiwi (*Actinidia deliciosa*), e — an adult on a catalpa leaf (*Catalpa bignonioides*), f — nymphs on common cocklebur (*Xanthium strumarium*); g — Italian striped bug *Graphosoma lineatum* in inflorescences of wild carrot (*Daucus carota*) (Belgorod Province, 2021); the green shield bug *Palomena prasina* (Moscow Province, 2018): h — an adult and a nymph on rosehip (*Rosa* sp.), i — a nymph on the rose flower (*Rosa* sp.), j — rose flowers damaged by *P. prasina*; k — red-legged shield bug *Pentatoma rufipes* (Belgorod Province, 2020).

In the former USSR, the southern green stink bug was known as a not-numerous inhabitant of natural ecosystems in the south of the Crimea and Transcaucasia which only occasionally caused damage to bean crops in Georgia [16]. However, since 2006 it has been recorded in the steppe regions of Krasnodar Krai as a significant pest on soybeans [58, 59]. In recent years, cases of mass colonization of *N. viridula* on vegetable crops, such as tomato (*Solanum lycopersicum* L.), pepper (*Capsicum annuum* L.), eggplant (*Solanum melongena* L.) have become more frequent, and damage to plants by the southern green stink bug is very significant [59, 60]. In Krasnodar Krai, this pest causes serious losses to farmers who have to complete the cultivation of tomatoes a month and a half earlier than they usually did [32]. In Stavropol Krai, since 2017, outbreaks of *N. viridula* have also taken place in greenhouses, where the pest damaged cucumber greens (*Cucumis sativus* L.) to a non-marketable state [N.I. Budynkov, Russian Research Institute of Phytopathology, personal communication). An increase in the harmfulness of the species has also been noted on tobacco plants (*Nicotiana tabacum* L.) [61].

Currently, the adaptation of *N. viridula* to new conditions in the south of Russia continues, there is an expansion to new host plants. The species is characterized by high fecundity, ability to form aggregations and multivoltinism (in favorable years it can produce up to three generations per season). To a greater extent, harmfulness manifests itself in dry hot weather [57, 59].

In the summer months of 2017-2019, the number of nymphs and adults of *N. viridula* in some farmlands of Adygea reached 40-50 specimen/m^2, which caused significant crop losses of tomatoes (up to 30-70%), sweet pepper and squash (*Cucurbita pepo* L. subsp. *ovifera* (L.) D.S. Decker), bean (*Phaseolus vulgaris* L.), cabbage (*Brassica oleraceae* L.), grape (*Vitis vinifera* L.), raspberry (*Rubus idaeus* L.) and other crops (62). In 2019, this was observed in the Crimea, where the shortage of tomatoes in household plots reached 70-90% (E.N. Zhuravleva, unpublished data).

The main reservoir of this phytophagous pest in the southern regions of Russia turned out to be not agricultural crops, but various plants of natural and ruderal flora on the outskirts of forests and along old forest belts, where the largest concentrations were observed on elderberry (*Sambucus nigra* L.) and wild privet (*Ligustrum vulgare* L.) [62]. In the humid subtropical zone, the population density of *N. viridula* is still substantially lower, but the patterns are the same [62].

In 2014, following the USA and European countries, a very dangerous alien pest of the East Asian origin – the brown marmorated stink bug *Halyomorpha halys* (Stel) (see Fig., d-f) [63, 64] arrived at Russia, on the Black Sea coast of the Caucasus. At the same time, the potential of the species for further settlement is assessed as very high not only by foreign researchers [65], but also by Russian scientists: it is assumed that the phytophagous pest can adapt on the territory of the European part of Russia, up to Kursk, Belgorod, Voronezh and Saratov regions [66].

Since 2016, this phytophagous pest has caused impressive crop losses of citrus fruits in the humid subtropics of Russia: primarily tangerine (*Citrus reticulata* subsp. *unshiu* (Marcow.) D. Rivera & al.), apple (*Malus domestica* Borkh.), pear (*Pyrus communis* L.), peach (*Prunus persica* (L.) Batsch), persimmon (*Diospyros kaki* Thunb.), fig (*Ficus carica* L.), hazelnut (*Corylus avellana* var. *pontica* (K. Koch) H.J.P. Winkl.), bean, tomato, sweet pepper, corn (*Zea mays* L.) and others [64]. During 6 or 7 years of its presence in the humid subtropics of Russia, the range of host plants damaged by this pentatomid has exceeded 100 species from many families. Among tree and shrub species, it is often found on cherry laurel (*Prunus laurocerasus* L.), southern catalpa (*Catalpa bignonioides* Walter), princess tree (*Paulownia tomentosa* Steud.), common mulberry (*Morus alba* L. and *Morus nigra* L.), common hazel (*Corylus avellana* L.), beech (*Fagus orientalis* Lipsky), lime tree (*Tilia begoniifolia* Steven), ash tree (*Fraxinus excelsior* L.), blackberry (*Rubus caucasicus* Focke and other species), and among herbaceous plants — on common cocklebur (*Xanthium strumarium* L.), thistle (*Cirsium* spp.), cockspur (*Echinochloa crus-galli* (L.) P. Beauv.), bittersweet (*Solanum dulcamara* L.) [67].

In other parts of the invasive range of *H. halys*, there is also an expansion of trophic connections of this species. Thus, for the first time it was noted as a pest of rice (*Oryza sativa* L.) in northern Italy in 2017 [68], and since 2018 — in California [69]. Significant damage by this species to kiwi (*Actinidia deliciosa* (A. Chev.) C.F. Liang & A.R. Ferguson) was recorded in Italy in 2018-2019 [70). Since 2019, that is 4 or 5 years after its appearance in Romania, *H. halys* begun to show increased harmfulness to garden and ornamental plants [71].

Serious concerns are caused by the ability of *H. halys* (as well as a number of other pentatomids) to carry the yeast fungus *Eremothecium coryli* (Peglion) Kurtzman (Ascomycota, Saccharomycetales), which can cause mass rot of tomato fruits [72] and lead to economically significant loss of hazelnut kernels [73].

In the Eastern Black Sea region, the main reservoirs of *H. halys* are natural plantings, where its number is immeasurably higher than in the agricultural sector and on ornamental plantings. Massive aggregations of the pest have been noted in protected natural areas (in the forests of Sochi National Park and the Caucasian State Natural Biosphere Reserve), where, according to modern legislation, the use of pesticides is unacceptable [62]. Thus, it is not possible to eradicate *H. halys*, although it is required according to the Federal Service for Veterinary and Phytosanitary Surveillance regulations regarding quarantine pests. Due to the high migration ability of this pest at the adult stage and its wide polyphagy, even a protection system coordinated between different departments is doomed to low efficiency. The only measure to control *H. halys*, which, with a well-coordinated organization, might give a positive result, is the manual collection of adults in places of their mass overwintering (attics of houses, sheds, timber warehouses, stacks of firewood, etc.) with subsequent immediate destruction [62]. However, such practice cannot solve the problem cardinally.

The extension of the growing season is one of the important consequences of the climate change, especially in temperate latitudes [26, 74]. This, in turn, may contribute to an increase in the number of generations realized by phytophagous insects during a year [36]. Thus, in the 1990s, it was shown that under the conditions of the forest-steppe of Belgorod Province, the Italian striped bug *Graphosoma lineatum* (L.) (see Fig., g) is usually univoltine but can give two generations per season only in the warmest years [75]. Two decades later, in 2019-2021, according to the observations in Shebekinsky District of this region, the bivoltinism of the species probably became normal. Mass emergence of adults was observed not in mid-July (as it was in the past), but almost a month earlier (in late June), and in late September there was a very high number of adults (especially on the ubiquitous wild carrot *Daucus carota* L.), which at the end of the growing season could hardly be like that with the development of only one generation per season (B.A. Borisov, unpublished data).

A frequent consequence of the steady increase in average temperatures is the better survival of many insects during overwintering, and in summer — an increase in fecundity, which strongly depends on the available amount of effective temperatures among poikilothermic organisms [34]. Probably, this can explain the increase in population density of some pentatomid species observed in different regions of Russia in recent years. According to the long-term observations in different districts of Moscow Province, the abundance of the previously relatively not-numerous, albeit common, native green shield bug *Palomena prasina* (L.) (see Fig., h-j) has increased many times in 2016-2020 both in household gardens (on bean *Phaseolus* L., broad bean *Vicia faba* L., black chokeberry *Aronia melanocarpa* (Michx.) Elliott, rose *Rosa* spp., tatarian dogwood *Cornus alba* L.), and on the edges of woodlands (on stinging nettle *Urtica dioica* L., dead-nettle *Lamium* spp., hemp-nettle *Galeopsis tetrahit* L., three-lobe beggarticks *Bidens tripartita* L., touch-me-not *Impatiens noli-tangere* L., burdock *Arctium lappa* L., raspberry, blackberry, rosehip *Rosa* spp., snowy mespilus *Amelanchier ovalis* Medik., rowan *Sorbus aucuparia* L., alder buckthorn *Frangula alnus* Mill., guelder-rose *Viburnum opulus* L.). Numerous symptoms of plant diseases have been noted in the places where this species is gathered: on raspberry — a strong development of viral infections transmitted by this bug (crinkle and chlorosis of leaves), on snowy mespilus — damage of berries by monilia, on roses — browning and drying of flowers impaled by the pest due to the development of bacteriosis (see Fig., j) [62].

In recent years, similar situations have been noted in Moscow Province with the native sloe bug *Dolycoris baccarum* (L.) (B.A. Borisov, unpublished data). In July 2020, in a number of farm fields throughout Belgorod Province, abundance of this species and of black-shouldered stink bug *Carpocoris purpureipennis* (De Geer) on ears of bread wheat (*Triticum aestivum* L.) was noticeably higher than of the "traditional" pest – the sunn pest *Eurygaster integriceps*. It is opposite to what had been usually observed (B.A. Borisov, unpublished data).

Also, in recent years, not only an increase in the harmfulness of *P. prasina*, *D. baccarum*, *Eurydema ornata*, *Aelia acuminata*, *Rhaphigaster nebulosa* (Poda) and *Graphosoma semipunctatum* (F.) (a species typical to the Mediterranean region) was noted, but also a high density and prevalence of these species in Azerbaijan [76].

The usual trans-Palearctic red-legged shield bug *Pentatoma rufipes* (L.) (see Fig., k), associated with trees and shrubs in forest ecosystems [8, 77-79), had not been practically mentioned in the world literature as an economically significant pest [16, 80], however, in recent years, in Europe it has become a serious pest of fruit trees (apple trees, pears) [79, 81]. Unlike many other pentatomids, this species obligately overwinters at the nymphal stage [79]. A noticeable increase in its population density in the Republic of Chuvashia and in Perm City in 2019 is likely to be associated with a softening of the overwintering conditions, which contributes to an increase in the survival rate of overwintering nymphs [82, 83].

An outbreak of mass reproduction of *P. rufipes* was also noted by us under the conditions of Belgorod Province in 2020. In summer, adults of this species were often found along the edges of woodlands on the leaves of the box elder (*Acer negundo* L.) and blackthorn berries (*Prunus spinosa* L.), which were significantly damaged by autumn. The mass reproduction of *P. rufipes* can be judged by the fact that, in various places on shady forest roads and trails, many dead adults of this species were noted in early September: their average number was about 4 specimen/m^2, but in some places it was 6-8 times higher. The reason for such a high death rate among adults remained unknown; only a few specimens out of hundreds collected were affected by the entomoparasitic fungus *Beauveria bassiana* s.l. (Ascomycota: Hypocreales: Cordycipitaceae) (B.A. Borisov, unpublished data).

A similar example is the species *Antiteuchus innocens* Engleman et Rolston, common in Central America, which had not previously considered a serious pest [84, 85], but in recent years, an increased population densities of this species have been recorded in the pine forests of Mexico; this lead to the weakening of trees [86]. The reasons for the increase of the abundance of *A. innocens* have not been identified yet.

As noted above, the most important factor in the appearance of phytophagous insects in new regions is the increasing intensification of transport (cars, by sea, railways, and air) due to which phytophagous pest can be inadvertently transported hundreds and thousands of kilometers. Then they have a chance to successfully establish and adapt to the local climate and vegetation, which are new to them, and to build up their populations. In the absence of natural enemies (predators, parasites, pathogens), outbreaks of mass reproduction and expansion of the invasive area often occur [62]. This way, *N. viridula* and *H. halys*, as well as many other pentatomid species, continue to spread around the world [28, 44, 87].

Brachynema germarii (Kolenati) is widely distributed from the Canary Islands and Mediterranean countries through Transcaucasia and Asian deserts to Mongolia and Northern China [4, 16]. In 2017, this species was discovered in Transbaikalia (Daurian State Nature Reserve) and in the south of Krasnoyarsk Krai [88], and in 2020 — in the Voronezh State Natural Biosphere Reserve [89]. It is assumed that this pentatomid could get to the south of Krasnoyarsk Krai with the personal belongings of air passengers from neighboring Tyva, and to Voronezh Province independently, as a result of natural spreading in the north-west direction under conditions of abnormally hot weather.

The mottled shield bug *Rhaphigaster nebulosa* (Poda) is widely distributed in central, southern and southeastern Europe, in the Caucasus and Transcaucasia, Kazakhstan, Turkey, Iran, Pakistan, Asia, and North Africa [4]. During the last two decades, there have been reports of expanding of the range of this species to the north in different parts of Europe [90, 91]. In Russia, until recently, *R. nebulosa* had never been found in Voronezh Province, however, in 2010-2020, 73 specimens of this bug were collected in the region along with five specimens of its obligate parasite – tachinid fly *Cylindromyia bicolor* (Olivier) (Diptera: Tachinidae) [92].

In recent years, the East Asian (Malaysia, Thailand, Cambodia, Vietnam, Laos, southern China, and Japan) polyphagous yellow-spotted stink bug *Erthesina fullo* (Thunberg) has made long-distance movements. Since 2017, it has been repeatedly found in Albania [93], and since November 2020 — in Brazil, near the port city of Santos [94]. The bug is dangerous for citrus fruits, pears, persimmons, common jujube (*Zizyphus jujuba* Mill.), Ceylon cinnamon tree (*Cinnamomum verum* J. Presl), pine trees (*Pinus* spp.) and other plants [95]. If this species manages to naturalize in the countries of southern Europe, then there is a risk of its invasion into the territory of Russia and acclimatization at least in the south of its European part.

Among pentatomids, the polyphagous African stink bug *Bagrada hilaris* (Burmeister), which came to the USA about 15 years ago, and later to Mexico, Chile and India, is also of concern. It is known to damage cabbage and other crops of the *Brassicaceae* family [96]. In Europe, it has already been recorded on Malta and on the Italian island of Pantelleria [97]. The species poses a great threat to the countries of the Mediterranean basin [97], and later to the southern regions of Russia.

Thus, it is possible to identify several of the most likely causes leading to the expansion of ranges, increase in population density, and harmfulness of pentatomids. First of all, this is the global climate change (most significantly, warming), which in many cases contributes to an increase in the number of generations realized during the growing season, expanding opportunities to adapt to conditions of higher latitudes, often contributing to an increase in insects' fecundity. Other reasons include polyphagous nature of pentatomids, their high migration ability at the adult stage, unintentional introduction as a result of the intensification of transportation of various goods and the development of tourism, the absence of natural enemies (predators, parasites, pathogens) in the recipient regions, low co-ordination of quarantine and plant protection systems. An urgent issue for further research and practice of plant protection is the development of measures for effective control of pest populations which pose a particular threat to crops, but at the same time have a high population density in natural ecosystems.

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EFFECTIVENESS OF NITROGEN-FIXING SYMBIOSIS OF GUAR (*Cyamopsis tetragonoloba*) WITH STRAINS *Bradyrhizobium retamae* RCAM05275 AND *Ensifer aridi* RCAM05276 IN POT EXPERIMENT

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Abstract

Legume plant guar (*Cyamopsis tetragonoloba* (L.) Taub.) is a source of guar gum, a complex of polysaccharides that is used in various industries. This crop is widely cultivated mainly in India and Pakistan, but in recent years there has been an increasing interest in the industrial cultivation of guar in the southern regions of Russia. One of the problems of introducing this culture into Russian agriculture is the absence in the soil of bacteria that can form symbiotic nodules on the roots of guar in the soil-climatic conditions of the Russian Federation. One of the problems of the introduction of this crop into the agriculture of the Russian Federation is the absence of bacteria capable of forming symbiotic nodules on guar roots under environmental conditions of Russia. In the present work, the first data on the efficiency of inoculation with nodule bacteria of guar growing in the soils of Russia were obtained. The aim of this work was to evaluate the effectiveness of promising rhizobial strains of guar *Bradyrhizobium retamae* RCAM05275 and *Ensifer aridi* RCAM05276 when growing plants in soils selected in the regions of the Russian Federation and not containing the corresponding nodule bacteria. To inoculate the seeds of guar variety Kubanskiy Yubileiny, inoculums in the form of aqueous suspensions of bacteria were used. Inoculums were obtained according to the standard procedure for the preparation of liquid biopreparations of nodule bacteria. The effectiveness of symbiosis was studied in a pot experiment with growing plants in sod-podzolic soil and chernozem. Inoculation with both strains resulted in active formation of nodules (about 20-40 nodules per plant), while no nodules were found on the roots of control uninoculated plants. The number of nodules per plant was maximal in the variants of inoculation with the strain *B. retamae* RCAM05275. The values of the total mass of nodules per plant were maximum in the variants of inoculation with the strain *E. aridi* RCAM05276 due to the formation of larger nodules. The nodules formed on lateral roots had a rounded irregular shape, pinkish color (evidence of the presence of leghemoglobin in them) and significantly varied in size. Both strains increased the biomass of shoots and the whole plant by about 70% when growing plants on sod-podzolic soil and chernozem, but did not affect the biomass of the roots. The inoculated plants had approximately the same nitrogenase activity regardless of the rhizobia strain and soil type. The specific nitrogenase activity (per nodule biomass) was approximately 2 times higher than in other variants when plants were inoculated with the strain *B. retamae* RCAM05275 in sod-podzolic soil. In all variants of the experiment, an approximately 1.4-fold increase in the total nitrogen content and a 3-4-fold increase in nitrogen accumulation in the shoots of inoculated plants were revealed. Thus, the first data on the efficiency of inoculation with nodule bacteria of the guar cultivated in Russian soils have been obtained. Both studied strains were able to form nitrogen-fixing symbiosis, which led to a significant increase in plant biomass and accumulation of nitrogen in shoots. The results showed the promise of further research on testing strains in field experiments in order to create biopreparations to improve the nitrogen nutrition of this crop.

Keywords: nitrogen fixation, guar, nodulation, symbiosis, *Cyamopsis tetragonoloba*

Guar *Cyamopsis tetragonoloba* (L.) Taub. is an important tropical legumes, the seeds of which contain guar gum used in the coal, oil, gas, food, textile, paper and cosmetic industries. Guar is widely cultivated in India and Pakistan, and also in Afghanistan, Kenya, Australia and the semi-desert regions of the USA [1]. Demand for guar gum is constantly growing, and in 2016, imports of guar gum to Russia exceeded 15 thousand tons [2]. Guar was brought to Russia in the mid-1920s [3], but did not find wide distribution due to insufficient knowledge about the technology of cultivation [4]. In recent years, interest in the commercial growing of guar in the Southern Russia has been growing [5]. A way to address the chellenge is searchinf for strains of nodule bacteria (rhizobia) capable of forming an effective nitrogen-fixing symbiosis with guar in the soil under climatic conditions of the Russian Federation and to procude novel biological preparations based on these microorganisms.

Seed inoculation with biopreparations of nodule bacteria provides intensive biological nitrogen fixation which enhances photosynthesis and increases the yield of legumes [6, 7]. The use of rhizobia for inoculation is especially important when cultivating legumes in new areas, the soils of which do not contain the necessary microsymbionts. For example, when trying to grow soybean *Glycine max* (L.) Merr. in geographic zones of Russia atypical for the species, nodules were practically not formed on the roots. Therefore, it was necessary to develop biological preparations and inoculate seeds with specific rhizobia [6]. Currently, to solve this problem, soybean seed producers provide their supply together with nodule bacteria biopreparations (https://kingsagriseeds.com/soybeans/).

Slowly growing root nodule bacteria of the genus *Bradyrhizobium* (family *Bradyrhizobiaceae*) are the main group of rhizobia entering into symbiosis with guar [8, 9]. In most cases, the strains were not identified to species level but many were close to *B. japonicum* [10, 11]. This species includes a very large and genetically heterogeneous group of soybean microsymbionts, as well as strains that nodulate different types of cowpea (*Vigna*), lupine (*Lupinus*), seradella (*Ornithopus*) and a wide range of leguminous plants of the genistoid complex [12-14].

Inoculation of guar with strains of *Bradyrhizobium* spp. has a positive effect on plant development, significantly increases the number of nodules (up to 79%), plant weight (up to 71%), root weight (up to 262%), seed yield (up to 53%), protein content (up to 33%), fiber (up to 26%), increases the total content of nitrogen and minerals [8, 12, 15-17]. The ability of guar to form symbiosis with fast growing rhizobia *Ensifer aridi* (family *Rhizobiaceae*), which also have a wide range of leguminous hosts from the subfamilies *Mimosoideae* and *Papilionoideae*, has recently been described [18]. The presence of representatives of the genera *Bradyrhizobium* and *Ensifer* among guar nodule bacteria is common to this plant, soybean and cowpea [19-21].

Along with the creation of new varieties and development of special agrotechnologies, including mineral fertilizing, successful introduction of guar in Russia requires the selection of effective microsymbionts to create biological preparations for the crop. In India, the use of mineral nitrogen fertilizers is limited by the high cost and low level of agricultural mechanization. Therefore, the high productivity of guar is achieved largely due to the presence of highly effective nodule bacteria strains in soils. It should be emphasized that in the regions of Russia where guar cultivation has recently begun (Krasnodar Territory, Crimea, Astrakhan and Volgograd regions, and Dagestan) no symbiotic nodules appear, as it is reportef by Rusian breeders.

Previously, we isolated guar nodule bacteria, studied their biodiversity, and as a result characterized slow-growing strains of the genus *Bradyrhizobium* [22] and fast-growing strains of the genus *Ensifer* (unpublished data).

The purpose of this work was to assess the capability of effective symbiosis in two promising strains of rhizobia (the genera *Bradyrhizobium* and *Ensifer*) with guar plants grown in pots with soil samples from different regions of Russia lacking complementary nodule bacteria.

Materials and methods. Nodule bacterium strains *Bradyrhizobium retamae* RCAM05275 [23] and *Ensifer aridi* RCAM05276 [24] isolated from guar nodules, were characterized in preliminary experiments, patented by the authors, and deposited (the Departmental Collection of Agricultural Useful Microorganisms, ARRIAM, St. Petersburg). For seed treatment with bacteria, an inoculum prepared in the laboratory as a liquid sample was used. The pure bacterial inoculum was placed in flasks with 250 ml of a semi-synthetic nutrient medium with (g/l) mannitol, 10; yeast extract 1; K_2HPO_4 0.5; $MgSO_4 \cdot 7H_2O$ 0.2; NaCl 0.1. The cultures were incubated at 28 °C on a shaker at 180 rpm for 3 days (*E. aridi* RCAM05276) and for 5 days (*B. retamae* RCAM05275). With standard asepsis precautions, the contents of the flask were transferred into a laboratory fermenter BIORUS 5L (BIORUS, Russia) containing 5 l of the same medium. The fermentation was performed at 28 °C with aeration of 1 l air $\cdot$ 1 l medium$^{-1} \cdot$ min^{-1} and stirring at 200 rpm for 3 days (*E. aridi* RCAM05276) or 4 days (*B. retamae* RCAM05275). Then the bacterial suspensions (inoculum) were placed in a sterile container and stored at room temperature (22-24 °C) for later use.

Seeds of the guar *Cyamopsis tetragonoloba* (L.) Taub. cv. Kuban Yubileiny (Vavilov All-Russian Institute of Plant Genetic Resources St. Petersburg) were scarified and surface sterilized in 98% H_2SO_4 for 30 min, washed with sterile tap water and germinated on filter paper in Petri dishes at 25 °C in the dark for 2 days. For the pot test, we used soddy-podzolic soil (Albic Retisol, Abruptic, Ochric, Pskov Province) and chernozem (Haplic Chernozem, Pachic, Voronezh Province) sampled in the summer of 2017. Soddy-podzolic soil was provided by the Pskov Research Institute of Agriculture and the Rodina state farm (57°50′44.2″ N, 28 12′03.7″ E), chernozem was obtained in the Kamennaya Steppe reserve (51°01′41.6″ N, 40 43′39.3″ E). Agrochemical parameters of dry soil were measured according to standard methods [25]. The soil was introduced into 400 g metal pots and fertilized with K_2HPO_4 (600 mg/kg). The germinated seeds were exposed to the inoculum for 1 h and planted (2 pots, 4 seedlings for each treatment). Plants were grown for 106 days in a phytoroom with a relative humidity of 60% at a 2-level light and temperature regimes, darkness, 18°C, 8 h for night, 400 $\mu lx \cdot m^{-2} \cdot s^{-1}$, 23 °C, 16 h for day. DNaT lamps (OOO SSZ Lisma, Russia) and L36W/77 FLUORA lamps (OSRAM Licht AG, Germany) were used for illumination. Soil moisture was maintained at the level of 60-70% of the total moisture capacity by regular watering while weighing the pots. On day 45, the soil was additionally fertilized with K_2HPO_4 solution (600 mg/kg).

At the end of the experiment, the plant roots together with the formed nodules were separated from the shoots, washed with tap water, placed in sealed plastic test tubes, and acetylene (5% of the tube volume) was added to measure biological nitrogen fixation by the acetylene method. To do this, after incubating the tubes at 25 °C in the dark for 1 h, the amount of ethylene was determined on a GC-2014 gas chromatograph with a FID detector and a SUS 2.0 m×3.0 mm (L×ID) column (Shimadzu Corporation, Japan). Analytical parameters: detector temperature +250 °C; nitrogen as a carrier gas; the nitrogen flow rate 70 ml/min; injector temperature +72 °C; column temperature +70 °C; analysis time — 5.0 min; ethylene retention time 1.20±0.01 min; acetylene retention time 2.00±0.01 min.

Roots were photographed using a PC1742 camera (Cannon, Japan) and nodules were photographed using a Stemi 508 stereomicroscope (Carl Zeiss, Germany).

The nodules were separated from the roots and cpunted for each plant. Shoots, roots, and nodules were dried at room temperature and weighed. The content of total nitrogen in the shoots was measured (a Kjeltec 8200 automatic analyzer, FOSS Analytical, Denmark) according to the manufacturer's standard method.

Statistical analysis of data was performed using STATISTICA v. 10 (TIBCO Software Inc., USA). Differences between mean values were assessed using one-way analysis of variance and Fisher's LSD test, and the equality of sample variances was checked using Levene's Test.

Results. The table shows agrochemical parameters of the soil samples we used in the experiment.

Agrochemical parameters of soils used in the experiment

Parameter	Sod-podzolic	Chernozem
Mass fraction of organic matter, %	2.4	8.8
Nitrogen total, %	0.22	0.38
Ammonia nitrogen, mg N/kg	25	37
Nitrate nitrogen, mg N/kg	9.6	26.3
Mobile phosphorus, mg P_2O_5/kg	85	121
Mobile potassium, mg K_2O/kg	60	155
Hydrolytic acidity, mmol/kg	29	18
The sum of absorbed Ca^{2+}, $Mg2^{+}$, Na^{+}, K^{+}, NH^{4+}, mmol/kg	60	372
pH_{H2O}	6.1	7.3
pH_{KCl}	4.9	6.2

Note. Soddy-podzolic soil and chernozem were selected in 2017, respectively, in Pskov (57°50′44.2″ N, 28°12′03.7″ E) and Voronezh (51°01′41.6″ N, 40°43′39.3″ E) regions. The mass fraction of organic matter was measured by Tyurin's method; total nitrogen by Kjeldahl method; ammonia nitrogen with Nessler's reagent (extraction with 2% KCl solution); nitrate nitrogen as per the disulfophenol method (extraction with 0.05% K_2SO_4 solution); mobile phosphorus by formation of phosphomolybdenum blue, mobile potassium by the flame photometric method; extraction from soddy-podzolic soil was carried out with 0.2 mol/l HCl, from chernozem with 10 g/l $(NH_4)_2CO_3$; hydrolytic acidity and the amount of absorbed bases were measured by the Kappen method using extraction with 1 N. $CH3COONa \cdot 3H_2O$ and 0.1 N. HCl, respectively.

The inoculation of guar with both strains led to the active formation of nodules (approx. 20-40 per plant, Fig. 1, A), while no nodules were found on the roots of the control plants. When growing plants on chernozem, the number of nodules formed by *B. retamae* RCAM05275 was 2.8 times higher than in plants grown on soddy-podzolic soil and inoculated with *E. aridi* RCAM05276. The total weigh of nodules per plant was maximum with *E. aridi* RCAM05276 on soddy-podzolic soil and chernozem, but the differences between the test options were not significant due to wide variation in this parameter (see Fig. 1, B).

It has previously been shown that nodulation in guar can be inhibited by high concentrations of available soil nitrogen (26). In the chernozem we used, the content of various forms of nitrogen was 1.5-2 times higher than in the soddy-podzolic soil (see Table). However, this did not lead to the inhibition of nodule formation, which indicates the ability of both partners to form symbiosis at different levels of soil nitrogen.

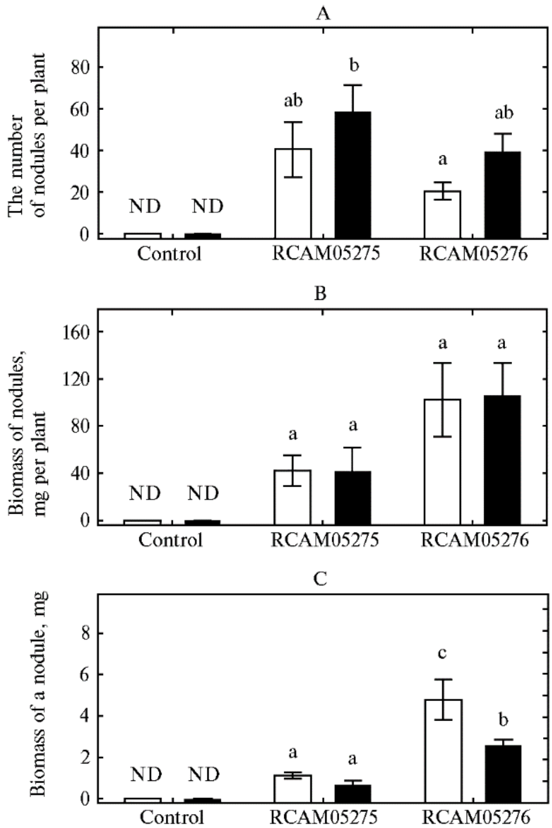

Fig. 1. The number of nodules (A) **and the dry biomass of nodules (B) per plant and the biomass of one nodule (C) on the roots of guar *Cyamopsis tetragonoloba* (L.) Taub. cv. Kuban Yubileiny:** control — without inoculation, RCAM05275 — inoculation with the strain *Bradyrhizobium retamae* RCAM05275, RCAM05276 — inoculation with the strain *Ensifer aridi* RCAM05276; white columns — sod-podzolic soil, black columns — chernozem. Vertical bars indicate mean error, ND — not detected. Statistically significant differences are marked with different Latin letters (n = 8, Fisher's LSD test, $p < 0.05$; lab experiment).

As compared to *B. retamae* RCAM05275, the strain *E. aridi* RCAM05276 can form nodules larger in size (see Fig. 1, C). The largest nodules in the strain *E. aridi* RCAM05276 were formed on soddy-podzolic soil. Information on the effect of soil type and composition on the size of symbiotic nodules and the role of the rhizobia strain in this dependence is very limited.

It is known that the plant controls the formation (number and biomass) and functioning (photosynthate influx and transport of nitrogen compounds) of nodules [27]. The number and biomass of formed nodules also vary significantly depending on the microsymbiont strain [6, 28, 29]. The processes of symbiosis formation depend on the physicochemical properties of the soil, such as the content of organic matter, nitrogen available to plants, acidity, and other factors [29, 30]. Aboriginal rhizospheric microorganisms, which modulate the hormonal status and supply of plants with nutrients and interact with introducers, have a great influence on the number of nodules formed [31]. The observed phenomenon of soil influence on nodule biomass can be associated with several of the listed factors and its explanation requires a more detailed study.

Both strains increased the biomass of shoots by approx. 70% when growing guar on soddy-podzolic soil and chernozem (Fig. 2, A), but did not affect the biomass of roots (see Fig. 2, B). As a result, the biomass of the entire inoculated plant in all variants of the experiment was also greater than in the control, by about 60-80% (see Fig. 2, C).

This is consistent with the literature data on the high responsiveness of guar to inoculation with nodule bacteria, which manifested itself in an increase in the biomass of the aboveground parts of plants and seed yield [8, 11, 13].

Figure 3, as an example, shows the appearance of the above-ground part, the root system and nodules of plants grown on soddy-podzolic soil on the day the experiment was completed.

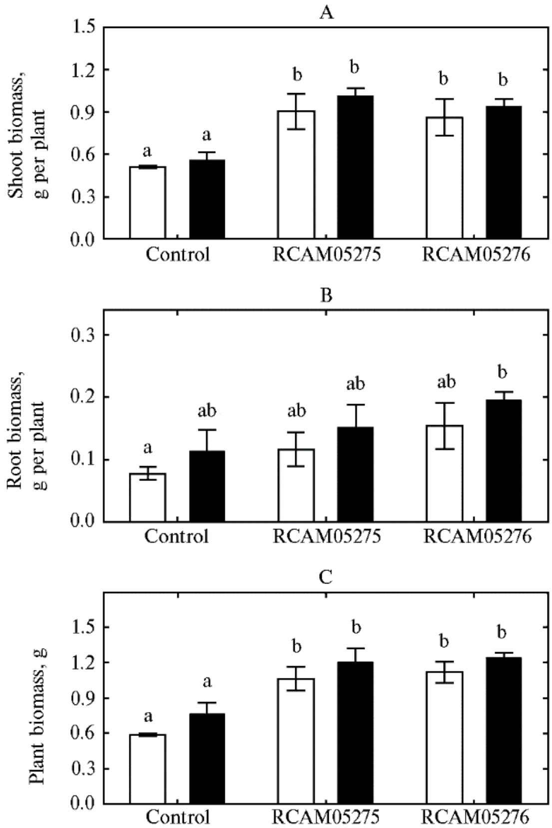

Fig. 2. Dry biomass of shoots (A), **roots** (B) **and total plant biomass** (C) **of guar *Cyamopsis tetragonoloba* (L.) Taub. cv. Kuban Yubileiny:** control — without inoculation, RCAM05275 — inoculation with the strain *Bradyrhizobium retamae* RCAM05275, RCAM05276 — inoculation with the strain *Ensifer aridi* RCAM05276; white columns — sod-podzolic soil, black columns — chernozem. Vertical bars indicate mean error, ND — not detected. Statistically significant differences are marked with different Latin letters (n = 8, Fisher's LSD test, $p < 0.05$; lab experiment).

The control plants were significantly inferior to the inoculated plants in terms of leaf height and area, and showed signs of chlorosis, probably due to nitrogen deficiency (Fig. 3, A). The roots were well developed, branched, but had no nodules (see Fig. 3, B). Both strains formed nodules on lateral roots, with single nodules and groups of closely spaced nodules occurring (see Fig. 3, C, E). The nodules of both strains had an irregular round shape and significantly varied in size (see Fig. 3, D, F). The pinkish color of the nodules indicates the presence of leghemoglobin which is necessary for atmospheric nitrogen fixation.

Fig. 3. Appearance of shoots (A), **roots** (B, C, E) **and nodules** (D, F) **of guar *Cyamopsis tetragonoloba* (L.) Taub. cv. Kuban Yubileiny plants grown on soddy-podzolic soil:** B — control without inoculation,

C, D — inoculation with *Bradyrhizobium retamae* strain RCAM05275, E, F — inoculation with *Ensifer aridi* RCAM05276 strain (lab experiment).

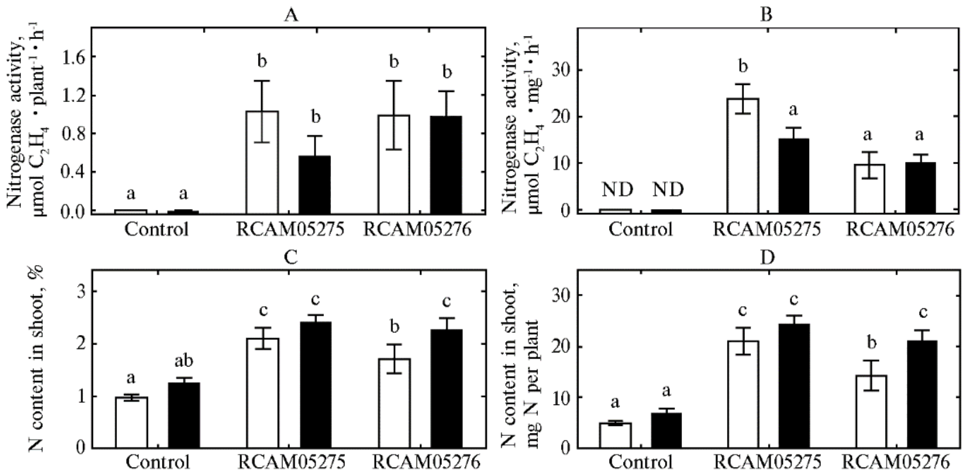

Fig. 4. Nitrogenase (acetylene reductase) activity (A), **specific nitrogenase activity per nodule biomass unit** (B), **content** (C) **and accumulation** (D) **of total nitrogen in guar *Cyamopsis tetragonoloba* (L.) Taub. cv. Kuban Yubileiny shoots:** control — without inoculation, RCAM05275 — inoculation with the strain *Bradyrhizobium retamae* RCAM05275, RCAM05276 — inoculation with the strain *Ensifer aridi* RCAM05276; white columns — sod-podzolic soil, black columns — chernozem. Vertical bars indicate mean error, ND — not detected. Statistically significant differences are marked with different Latin letters ($n = 8$, Fisher's LSD test, $p < 0.05$; lab experiment).

The inoculated plants had approximately the same nitrogenase activity regardless of the rhizobia strain and soil type (Fig. 4, A). But the specific nitrogenase activity, expressed per unit of nodule biomass, was 1.5-2.4 times higher in soddy-podzolic soil when plants were inoculated with B. etamae RCAM05275 than in other variants (see Fig. 4, B). It is likely that the relatively low biomass of nodules (see Fig. 1, B) was compensated by an increase in the efficiency of the nitrogen fixing system with the participation of the B. retamae RCAM05275 strain. Strain comparisons and measurement of nitrogen fixation activity were not performed in these studies, but nodule mass was previously shown to correlate with nitrogen accumulation and yield when comparing 50 guar genotypes [32]. Little is known about the level of nitrogenase activity of guar nodules, but the values obtained by us were comparable with the data on the measurement of nitrogenase activity in soybean inoculated with various strains of the genera Bradyrhizobium and Ensifer [33, 34].

The effective functioning of the symbiosis was also indicated by an increase in the total nitrogen content by about 1.4 times (see Fig. 4, C) and nitrogen accumulation by approx. 3-4 times (see Fig. 4, D) in the shoots of inoculated guar plants. These effects manifested themselves in all variants of the experiment with a minimum value during the inoculation of plants grown on soddy-podzolic soil with the E. aridi RCAM05276 strain. A significant increase in the content and accumulation of nitrogen in guar plants as a result of inoculation with nodule bacteria has been repeatedly described [8, 11, 12, 35]. It was also shown that strains of the genus Bradyrhizobium fixed nitrogen more actively in symbiosis with soybean than strains of the genus *Ensifer* [34]. In our experiments, B. retamae RCAM05275 was not inferior to the *E. aridi* RCAM05276 strain in terms of the measured parameters of symbiosis, and on soddy-podzolic soil it increased the content and accumulation of nitrogen in plants by 26% compared with the increase in these parameters under the influence of the *E. aridi* RCAM05276 strain (see Fig. 4, C, D). On average, for all variants of the experiment, the biomass of an individual nodule negatively correlated with the nitrogen content in the shoots ($r =$ 0.98; p = 0.019; $n = 4$), which indicates a higher efficiency of small nodules formed by the strain B. retamae RCAM05275.

Thus, both strains, *B. retamae* RCAM05275 and *E. aridi* RCAM05276, can form effective symbiosis with guar when growing plants in soddy-podzolic soil and chernozem, selected in different regions of the Russian Federation and not containing complementary nodule bacteria. The strains were similar in terms of nodule formation and symbiosis efficiency. However, the characteristic features of strains in interaction with guar plants were also revealed, which was expressed in differences in the number of nodules, specific nitrogenase activity, and intensity of nitrogen supply to plants. Our results showed the promise of further testing of the studied strains in field experiments in order to create biological preparations to improve the nitrogen nutrition of guar plants.

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EVALUATION OF THE BIOCONTROL EFFICACY OF *Serratia proteamaculans* AND *S. liquefaciens* ISOLATED FROM BATS GUANO PILE FROM A SUBTERRESTRIAL CAVE (GREECE)

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Abstract

Members of the genus *Serratia* are of great research interest because they are almost ubiquitous and exhibit emulsifying, surfactant, antifouling, antitumor and antimicrobial properties. Water is a natural habitat for several species of serrations. This paper reports on the first isolation of *S. proteamaculans* from bats guano. The aim of the present study is to evaluate the biocontrol activity of *Serratia* strains isolated from bats guano pile from a subterrestrial cave of Thessaly region (Aeolia), Greece. *Serratia* strains initial designated as strains Sl2, Sl4 and were able to ferment glucose (D-glucose), other carbohydrates (i.e. D-mannitol, D-mannose), and saccharose/sucrose as a source of carbon and sugars. Both strains have an optimal growth at 28 °C whereas strain Sl4 were able to grow and at 4 °C. Bacteria strains Sl2 and Sl4 were classified within the *Serratia liquefaciens* group by the VITEK® 2 system (bioMerieux SA, France) and were accurately identified at the species level by MALDI-TOF MS (bioMerieux SA, France). MALDI-TOF MS classified Sl2 strain as *S. proteamaculans* and Sl4 strain as *S. liquefaciens*. To the best of our knowledge, this paper is the first to report the detection and classification in detail of the *S. proteamaculans* in bat guano. Both *Serratia* strains produced prodigiosin at 28 °C with optimum prodigiosin production recorded 72 h after incubation. Further the antifungal activity of *S. liquefaciens* and *S. proteamaculans* strains were investigated in vitro against plant pathogenic fungi (*Fusarium oxysporum*, *Alternaria alternata*, *Botrytis cinerea*, *Sclerotinia sclerotiorum* and *Rhizoctonia solani*). This is the first report that *S. liquefaciens* and *S. proteamaculans* strains isolated from bat guano were able to produce freely diffusible compounds with fungistatic activity in vitro. Studies on the interaction between pathogen and bacteria confirmed the biocontrol efficacy of both *Serratia* strains (*S. liquefaciens* and *S. proteamaculans*).

Keywords: *Serratia* spp.*,* bat guano, subterranean aquatic environment, secondary metabolites, biocontrol.

Serratia species, which includes up to 18 species, can be found in several different environments [1]. Strains of the genus *Serratia* have been isolated from water, soil, plants, and animals [2]. In mammals, *Serratia* strains have been associated with infections such as mastitis in cattle, conjunctivitis in equine, septicaemia in foals, goats, and pigs, but have also been associated with several clinically healthy individuals [3]. *S. liquefaciens* and *S. marcescens* have been reported as opportunistic pathogens for the chiropteran species [4].

Serratia liquefaciens group consists of the species *S. liquefaciens*, *S. proteamaculans*, and *S. grimesii* [3]. Strains of the *S. liquefaciens* group predominantly cause sepsis and bloodstream infections via contaminated clinical equipment and blood components [5]. In 1980, the "Approved list of Bacterial Names" listed *S. liquefaciens* and *S. proteamaculans* as separate species [6]. Although probably rarely reported in clinical samples due to inability to easily discriminate between group species, *S. proteamaculans* has been shown to cause human disease [7].

Water appears to be a natural environment for several species, including *S. marcescens*, *S. fonticola*, *S. grimesii*, *S. liquefaciens*, *S. plymuthica*, *S. rubidaea*, and *S. ureilytica* [8]. *S. marcescens*, *S. liquefaciens*, *S. proteamaculans*, *S. grimesii*, and *S. plymuthica* were found in river water in one study, with the predominant species being *S. marcescens* followed by *S. liquefaciens* [3]. *S. marcescens* subsp. *sakuensis* was originally isolated from the suspended water of a wastewater treatment tank in Japan [9]. *Serratia* species are also associated with animals and cause important animal diseases. *S. marcescens* was described in 1958 as a cause of illness in animals, when part of a dairy herd was diagnosed with mastitis. There are many other reports of colonization or disease caused by *Serratia* species in animals, including but not limited to reptiles, rodents, birds, chicks, goats, pigs, fish, and horses [8].

Members of the *Serratia* genus are gaining increased scientific interest as they have been shown to exhibit emulsifying, surface, antifouling, antitumour and antimicrobial activity [10, 11]. Literature shows that the ubiquitous nature of this genus is due to the synthesis of numerous extracellular products, including exoenzymes, nucleases and secondary metabolites that aid in the adaption of *Serratia* to harsh environmental conditions [12] including prodigiosin. Certain strains of *S. marcescens*, *S. rubidaea*, and *S. surfactantfaciens* produce prodigiosin and show antibacterial and antifungal activity [2, 10].

Plant pathogenic fungi are responsible for severe losses of agriculture worldwide. An effective approach is to use chemical fungicides, to control the spread of fungi plant diseases, which have been applied to several fruits and other crop species. The rising threat of fungicide resistance in plant e.g. the grey mold fungus *Botrytis cinerea* resistance development is well-known. *B. cinerea* is notorious as a 'high risk' organism for rapid resistance development and the introduction of new fungicide classes for grey mold control was always followed by the appearance of resistance in field populations [13]. Under these circumstances, there is a growing need for identifying alternatives to fungicides in the prevention and treatment of microbial infections.

Base to above, the primary aim of this study was to examine bat guano from a subterrestrial aquatic ecosystem, for *Serratia* isolates capable of presenting biocontrol activity against plant pathogenic fungi.

Materials and methods. The Malaki cave is located in Thessaly (Aeolia), Greece (lat. 48°28′36″N, 20°29′09″E, alt. 339 m a.s.l.).

Gram-negative bacteria were isolated from bats guano. NA (Nutrient Agar), Potato Dextrose Agar (PDA), and MacConkey agar (MCA, Oxoid Limited, Great Britain) were used for routine isolation of bacteria. We used quadrant streak method, and agar plates were incubated at 22 °C for 2 days [1].

VITEK® 2 and MALTI-TOF MS (bioMerieux SA, France) were used to identify bacterial isolates at the species level [14, 15]. Single colonies on the nutrient agar slant were selected and suspended in 2.5 ml of 0.45% sterile saline to adjust the bacterial suspension to a 0.5 McFarland turbidity standard using a densitometer (bioMerieux SA, France) according to the manufacturer's instructions. Each bacterial suspension was prepared within 30 minutes of inoculation into the gram-negative (GN) cassette for identification by VITEK® 2. The biochemical test array of the GN cassette is presented in Table 1. The analysis was performed using VITEK® 2 software version 07.01 (bioMerieux SA, France) [16]. Higher probability than 80 % with a sufficient profile was considered a satisfactory identification within the possible identification spectrum of species or genera (taxa) by the VITEK® 2 [15, 16].

Prior to MALDI-TOF MS measurement, bacterial isolates were freshly inoculated on PCA (Oxoid Limited, UK) and cultivated for 24 h at 28 °C. The common direct transfer protocol (commonly referred to as whole-cell or intact-cell measurement) was followed to obtain mass spectra. Briefly, ~ 0.1 mg of cell material was directly transferred from a bacterial colony or smear of colonies to a MALDI target spot. After drying at laboratory temperature, sample spots were overlaid with 1 μl of matrix solution (10 mg/ml α-cyano-4-hydroxycinnamic acid in 50% acetonitrile and 2.5% trifluoroacetic acid). To determine mass spectra generation reproducibility, all cultures were cultivated independently four times (biological replicates); each measurement was carried out in triplicate. MS analysis was performed on an Autoflex MALDITOF mass spectrometer (Bruker Daltonics, Germany) using Flex Frontiers [16] with dereplication (Recurrent Bacterial Isolates Control 3.4 software, Bruker Daltonics, Germany): calibration was carried out with the use of the Bacterial Test Standard (Bruker Daltonics, Germany) (http://www.bruker.com/jp/products/mass-spectrometry-andseparations/literature/literatureroom.html?eID=dam_frontend_ push&stream=1&docID=58883). All MS spectra were measured automatically using Flex Control software (Bruker Corporation», USA, Germany) according to the standard measurement method for microbial identification. Specifically, our set-up values in linear positive mode were as follows: ion source 1 voltage, 20 kV; ion source 2 voltage, 19 kV; lens voltage, 6.5 kV; mass range, 2-20 kDa; the final spectrum was the sum of 10 single spectra, each obtained by 200 laser shots on random target spot positions. With regard to the functioning of MALDI-TOF MS, by which +1 ions are predominantly generated and detected, Da is used as a unit of m/z throughout the study. For bacterial classification using BioTyper 3.1 software (Bruker Daltonics, Germany) equipped with MBT 6903 MPS Library (released in April 2016), the MALDI Biotyper Preprocessing Standard Method and the MALDI Biotyper MSP Identification Standard Method adjusted by the manufacturer (Bruker Daltonics, Germany) were used [15, 16].

A VITEK® 2 GN identification card was used (the VITEK® 2 system, bioMerieux SA, France) [17] was used to assign the *Serratia proteamaculans* (Sl2) and *Serratia liquefaciens* (Sl4) strains to the *Serratia liquefaciens* group.

The *Serratia* strains (Sl2, Sl4) isolated from bat guano identified as *S. proteamaculans* (Sl2 strain) and *S. liquefaciens* (Sl4 strain) using a MALDI-TOF instrument, were screened for prodigiosin production on MacConkey's medium. Plates were incubated at 28 °C for 24, 48, and 72 h and screened for *Serratia* colonies with hyperpigmentation. The pigment producing strains (hyperpigmentation) were detected by appearance of pink-red growth.

Five different fungal strains causing plant disease were used in this study to assess antifungal properties of the *Serratia* strains. The fungal strains *Fusarium oxysporum*, *Alternaria alternata*, *Botrytis cinerea*, *Sclerotinia sclerotiorum*, and *Rhizoctonia solani* were isolated from affected tomato plants in Central Greece during earlier study [18, 19], with techniques described in [20, 21].

The isolated *Serratia* strains (*S. liquefaciens* and *S. proteamaculans*) were screened in vitro by dual culture techniques for the presence of antagonistic activity against the five different fungal species, *F. oxysporum* (BFI 2550), *A. alternata* (BFI 2596), *B. cinerea* (BFI 1952), *S. sclerotiorum* (BFI 2529), and *R. solani* (BFI 2531) obtained from Benaki Phytopathological Institute (BFI) as described by [22]. Specifically, in PDA plate, a 40 mm streak was made from 24h culture of bacteria 30 mm away from the centre of a petri dish. A 5 mm agar plug from a 5-day old fungal culture was placed at the centre of the petri dish with the test bacterial strain. Plates were incubated at 25 °C for 5 days and monitored for zone of inhibition daily. The mycelial radial growth (diameter) of the plant pathogens was measured. The features of the manifestation of antifungal activity were examined. All treatments were performed in eight replications.

Data were analysed using the Minitab statistical package (https://www.minitab.com/en-us/support/downloads/). Analysis of variance was used to assess antagonistic effect, the results are shown in an excel graph plotted the mean/treatment (*M*) and the standard error of differences between means (±SEM).

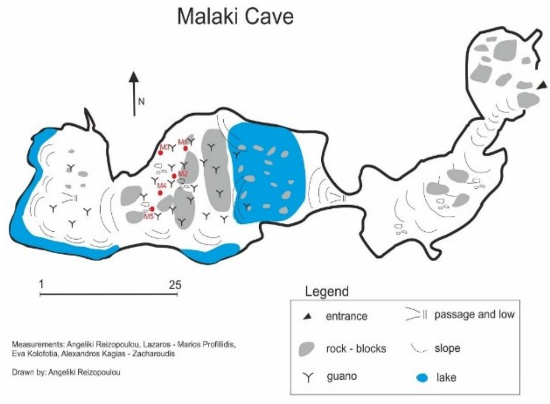

Fig. 1. Cave mapping (the Malaki cave, Thessaly, Aeolia, Greece; lat. 48°28′36″N, 20°29′09″E, alt. 339 m a.s.l.). Red dots indicate sampling sites close to indigenous bat nests. Drawn by Angeliki Reizopoulou.

Results.. Malaki cave is a small semi light limestones subterrestrial cave (Fig. 1). Malaki cave environment is being separated into three zones I) a twilight zone close to the entrance, II) a middle zone with scarce light and varying temperatures, and III) the deep zone of total darkness and a steady temperature (17 °C) throughout the year. In Cave's deep zone, there is a small subterrestrial lake, and several large bat guano piles. In cave's deep zone the main species fauna found are the genus *Miniopterus*, *Myotis*, and *Rhinolophus* [23].

Serratia strains initially designated as Sl2 and Sl4 were able to ferment glucose (D-glucose), utilize maltose, trehalose, other carbohydrates (i.e., D-mannitol, D-mannose), and saccharose/sucrose as a source of carbon and sugar (Table 1). Both strains have an optimal growth at 28 °C whereas strain Sl4 can also grow at 4 °C.

All bacterial strains with negative straight rod morphology, capable of growth on MacConkey Agar were correctly identified to the species level (99.9% probability) by the VITEK® 2 system using VITEK 2 GN ID card (see Table 1). VITEK 2 GN ID card classified both strains (Sl2 and Sl4) as *Serratia liquefaciens* group. The direct identification reporting time of VITEK® 2 ranged from 4.17 h to 4.65 h for all isolates. Furthermore, all strains identified using the VITEK® 2 were classified to the species level (99.9% confidence level) with MALDI-TOF MS system as *S. proteamaculans* (Sl2) and *S. liquefaciens* (Sl4). It is clear from the results of MALDI-TOF MS analysis that the confidence value of 99.9% as presented is beyond doubt evident of the clear distinction and presence of *S. proteamaculans* and *S. liquefaciens* strains.

1. Evaluation of VITEK® 2 GN ID Card for rapid identification of gram negative bacteria isolated from bats guano (Malaki cave, Thessaly, Aeolia, Greece; lat. 48°28′36″N, 20°29′09″E, alt. 339 m a.s.l.)

Test	Mnemonic	Amount	Strain *Serratia*	
			Sl2 (*S. proteamaculans*)	Sl4 (*S. liquefaciens*)
Ala-Phe-Pro-arylamidase	APPA	0,0384 mg	–	–
Adonitol	ADO	0,1875 mg	–	–
L-pyrrolydonyl arylamidase	PyrA	0,018 mg	+	+
L-Arabitol	1ARL	0,3 mg	–	–
D-Cellobiose	dCEL	0,3 mg	–	–
Beta-galactosidase	BGAL	0,036 mg	+	+
H_2S	H_2S	0,0024 mg	–	–
Beta-N-acetyl-glucosaminidase	BNAG	0,0408 mg	+	+
Glutamyl arylamidase pNA	AG LTp	0,0324 mg	–	–
D-glucose	dGLU	0,3 mg	+	+

Continued Table 1

Gamma-glutamyl-transferase	GGT	0,0228 mg	+	–
Fermentation/glucose	OFF	0,45 mg	+	+
Beta-glucosidase	BGLU	0,036 mg	+	+
D-maltose	dMAL	0,3 mg	–	–
D-mannitol	dMAN	0,1875 mg	+	+
D-mannose	dMNE	0,3 mg	+	+
Beta-xylosidase	BXYL	0,0324 mg	–	–
Beta-alanine arylamidase pNA	BAlap	0,0174 mg	–	–
L-proline arylamidase	ProA	0,0234 mg	+	+
Lipase	LIP	0,0192 mg	–	–
Palatinose	PLE	0,3 mg	+	–
Tyrosine arylamidase	TyrA	0,0276 mg	+	+
Urease	URE	0,15 mg	–	–
D-sorbitol	dSOR	0,1875 mg	+	+
Saccharose/sucrose	SAC	0,3 mg	+	+
D-tagatose	dTAG	0,3 mg	–	–
D-trehalose	dTRE	0,3 mg	+	+
Citrate (sodium)	CIT	0,054 mg	+	+
Malonate	MNT	0,15 mg	–	–
5-keto-D-gluconate	5KG	0,3 mg	+	+
[-Lactate alkalinisation	1LATk	0,15 mg	+	+
Alpha-glucosidase	AGLU	0,036 mg	+	–
Succinate alkalinisation	SUCT	0,15 mg	+	+
Beta-N-acetyl-galactosaminidase	NAGA	0,0306 mg	+	+
Alpha-galactosidase	AGAL	0,036 mg	–	+
Phosphatase	PHOS	0,0504 mg	+	–
Glycine arylamidase	GIyA	0,012 mg	–	N/A
Ornithine decarboxylase	ODC	0,3 mg	+	+
Lysine decarboxylase	LDC	0,15 mg	+	+
Decarboxylase Base	ODEC	N/A		
L-histidine assimilation	1H1Sa	0,087 mg	–	–
Coumarate	CMT	0,126 mg	+	+
Beta-glucoronidase	BGUR	0,0378 mg	–	–
O/129 resistance (comp. *vibrio*)	0129R	0,0105 mg	+	+
Glu-Gly-Arg-arylamidase	GGAA	0,0576 mg	+	+
L-malate assimilation	1MLTa	0,042 mg	–	–
ELLMAN	ELLM	0,03 mg	–	–
[-Lactate assimilation	1LATa	0,186 mg	–	–

N o t e. «–» or «+» mark gives the absence or presence of the corresponding trait, N/A — not applicable. The data for Sl2 has been reported earlier (Michail G., Reizopoulou A., Vagelas I. First report of *Serratia* species isolated from subterranean cave aquatic environment. *International Research Journal of Engineering and Technology*, 2020, 07(12): 1776-1780).

The microorganism identification by MADLI-TOF is based on four commercial systems and their databases: I) the MALDI Biotyper (Bruker Daltonics, Bremen, Germany); II) the Spectral ARchive and Microbial Identification System (SARAMIS™) (AnagnosTec, Potsdam, Germany); III) the Andromas (Andromas, Paris, France) and IV) the Vitek MS (bioMйrieux SA, Marcy l'Etoile, France). Each of the systems includes a MALDI-TOF instrument from either Bruker Daltonics or Shimadzu Corporation (Japan), and the most installed in routine laboratories systems are the MALDI Biotyper and the Vitek MS, which are the FDA-cleared platforms [24]. The systems differ in databases, identification algorithms, and instrumentation [25]. In daily laboratory practice, MALDI-TOF is used for bacterial or fungal identification from colonies grown on solid media. Up to now the procedure has been used for the identification of Gram-negative and positive rods, Gram-positive cocci, fastidious organisms, like mycobacteria, Nocardia and other actinomycetes, anaerobic bacteria, yeasts and filamentous fungi [26-29]. According to the data the identification rates of genus are extremely high (97-99%) and varies from 85% to 97% at the level of species [30-35].

Both microorganisms (*S. liquefaciens* and *S. proteamaculans*) appeared as lactose fermenters on MacConkey's medium (Fig. 2, A). *Serratia liquefaciens* can ferment lactose rapidly compared to *S. proteamaculans*. *S. liquefaciens* colonies are circular, entire, convex, medium, shiny red and opaque whereas *S. proteamaculans* colonies are circular, filamentous, convex, small, orange-red and opaque (Fig. 2, B). *S. liquefaciens* and *S. proteamaculans* colonies produced a clear reddish-orange (orange halo) colonies suggesting prodigiosin pigment production (see Fig. 2, B). The optimum prodigiosin production where obtain 72 h after incubation at 28 °C (Table 2).

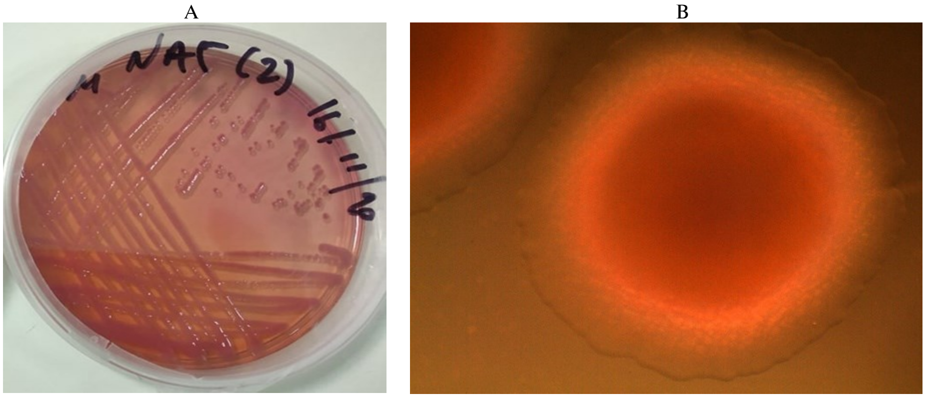

Fig. 2. Colonies of *Serratia liquefaciens* group bacteria isolated from bat guano (Malaki cave, Thessaly, Aeolia, Greece; lat. 48°28′36″N, 20°29′09″E, alt. 339 m a.s.l.) **after incubation on the MacConkey agar at 28 °C for 72 h:** A — lactose fermentation, B — pigmentation (prodigiosin production).

2. Effect of incubation time on prodigiosin production of *Serratia* strains isolated from bat guano (Malaki cave, Thessaly, Aeolia, Greece; lat. 48°28′36″N, 20°29′09″E, alt. 339 m a.s.l.)

Strain	24 h	48 h	72 h
S. proteamaculans	+/–	++	+++
S. liquefaciens	+/–	+	++

Note. Bacteria were incubated on the MacConkey agar at 28 °C. The mark +/– stands for very low pigment production, + for an enhanced pigment production; + for low pigment production, ++ for medium pigment production, and +++ for high pigment production.

S. proteamaculans and *S. liquefaciens* isolates exhibited significant antagonism results against *F. oxysporum*, *A. alternata*, *B. cinerea*, *S. sclerotiorum*, and *R. solani*. In details, *Serratia* species (*S. liquefaciens* and *S. proteamaculans*) were found to limit the colony growth of *F. oxysporum*, *A. alternata*, *B. cinerea*, *S. sclerotiorum*, and *R. solani* to a considerable extent (Fig. 3).

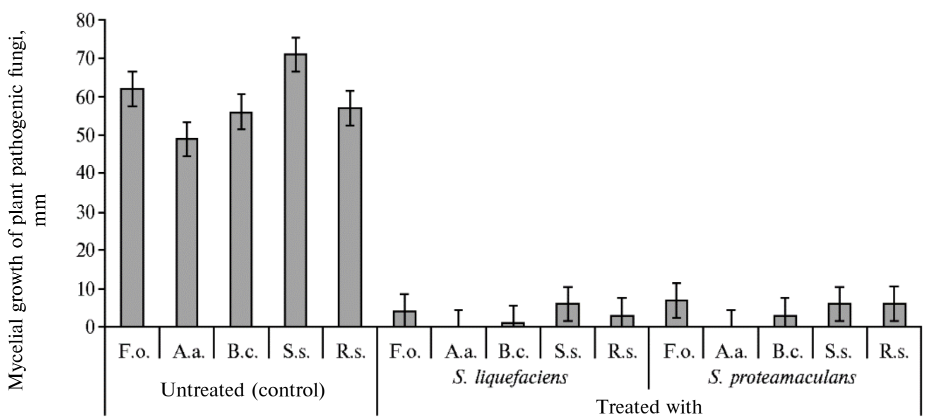

Fig. 3. Mycelial growth (mycelium diameter in mm) **of plant pathogenic fungi *Fusarium oxysporum*** (F.o.)**, *Alternaria alternata*** (A.a)**, *Botrytis cinerea*** (B.c.)**, *Sclerotinia sclerotiorum*** (S.s.)**, and *Rhizoctonia solani*** (R.s.) **treated in dual cultures with *Serratia liquefaciens* or *S. proteamaculans* isolated from bat guano** (Malaki cave, Thessaly, Aeolia, Greece; lat. 48°28′36″N, 20°29′09″E, alt. 339 m a.s.l.) (n = 17, M±SEM; PDA, 25 °C, 5 days).

Both bacterial *Serratia* species (*S. liquefaciens* and *S. proteamaculans*) isolated from bat guano pile showed significant antifungal activity against *A. alternata* in vitro compared to the untreated fungi plates.

In this research, we took samples of bat guano from different places from a subterrestrial Karst aquatic ecosystem which is the nesting place of several bat species. Bacteria that were isolated were grown on Nutrient agar, PDA and MacConkey agar. From these samples we cultured, isolated, and identified bacteria of the *Serratia liquefaciens* group using VITEK® 2 identification system. Using Matrix-Assisted Laser Desorption Ionization—Time of Flight Mass Spectrometry (MALDI-TOF MS) typing we have been able to classify the isolates of *S. liquefaciens* group to *S. liquefaciens* and *S. proteamaculans.* The latest breakthrough in identification of pathogens and determination of susceptibility are the techniques of mass spectrometry. Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF MS) mass spectrometry is an easy to use, rapid, reliable, economical, and environmentally friendly methodology that has revolutionized pathogen identification and detection of antimicrobial susceptibility/resistance in clinical settings [36]. On MacConkey agar all strains after 48h of incubation, produced the prodigiosin pigment as described by [37].

The *Serratia* strains isolated and then identified by VITEK® 2 identification system, were classified as *Serratia liquefaciens* group with four phenotypic characteristics difference between them as presented in Table 1. These characteristics (see Table 1) that were positive are the Alpha-Glucosidase, Palatinose, Gamma-Glutamyl, and the Phosphatase biochemical reaction. After the use of MALDI-TOF MS these characteristics distinguish the *S. proteamaculans* strain. *S. liquefaciens* isolate showed negative reactions to these characteristics. MALDI-TOF MS can be used for the identification of species in the *Serratia liquefaciens* group if necessary as it has been applied before with other *Serratia* species nosocomial outbreaks [14, 38]. In our study, we cultured from bat guano similar bacterial species specified as *Serratia liquefaciens* group. *S. liquefaciens* and *S. marcescens* have been described as opportunistic pathogens for many European bat species [4] but so far there has been no conclusive evidence of *S. proteamaculans* presence in either bat guano or as a part of their bacterial microflora. To the best of our knowledge, this is the first report of the presence of *S. proteamaculans* in bat guano.

Ever since its first applications for identification purposes [39, 40], MALDI-TOF MS has been proposed as a promising alternative for the dereplication of recurrent bacterial isolates [41-43] and has been used as a cost- and time-effective alternative to 16S rRNA gene sequencing [44-48]. MALDI-TOF MS-based identification of microorganisms involves the generation of mass spectra from whole-cell material or extracted intracellular content which are then matched to known database references [49, 50].

Newman et al. [51], in their recent research in caves of New Mexico, USA, presented no evidence of isolation nor identification of any *Serratia* species. Their research was based on bacteria isolation in TSA, blood agar, and bat guano medium (BGM) or by PCR amplification of 16s rRNA gene. They examined both fresh and decaying bat guano but did not find any *Serratia* spp. regardless finding different bacterial taxa on fresh and decaying guano [51]. According to our results, our findings show that there are different bacterial strains in bat microbiome and/or in guano between Europe's [4, 52, 53], India's [54, 55], America's [51] continent indigenous bats. Both European and Indian research in local bats guano revealed the presence of *Serratia* spp. but were not present in America's bat microbiome.

Serratia marcescens B4A, a novel *Serratia* strain, produces potent antifungal compounds and inhibits the growth of insects and plant pathogens such as *R. solani* and *A. raphanin* [56]. *Serratia* spp. also, are responsible for the production of secondary metabolites such as siderophores and phytohormone and shield the plants against pathogenic infections [56]. Some *Serratia* strains produce the halogenated secondary metabolite pyrrolnitrin which is a promising agricultural fungicide [56, 57]. *Serratia* species produce essential compounds and enzymes such as nucleases, chitinases, lipases, proteases, amylases, serralysin, and haemolysin [56]. There are quite a few studies regarding the important role of *Serratia* species as bio-control agents in agricultural crops management including strawberry, cauliflower, and olives. *S. plymuthica* A30 presents exceptional activity against the pathogenic bacteria *Dickeya solani* that cause blackleg and soft rot in potatoes [58-60].

In our study, both *Serratia* strains (*S. liquefaciens* and *S. proteamaculans*) were able to produce the red pigment prodigiosin and display significantly activity against *F. oxysporum*, *A. alternata*, *B. cinerea*, *S. sclerotiorum*, and *R. solani*. Literature shows that prodigiosin compound, that plays a role in biology not clearly clarified, has been produced by many *Serratia* species. Prodigiosin (2-methyl-3-pentyl-6-methoxyprodiginine) is a red-colored heterocyclic secondary metabolite that belongs to the class of tripyrrole compounds [61]. Prodigiosin appears in the later stages of bacterial growth acting as an overflow production of secondary metabolites with broad-spectrum antimicrobial activity [11]. The biosynthesis of prodigiosin is controlled by numerous environmental and physiochemical factors including temperature, oxygen and pH with maximum production yields achieved in the absence of light [61]. Other species within the *Serratia* genus such as *Serratia plymuthica, S. rubidaea and S. nematodiphila* are also capable of producing the non-diffusible red pigment, prodigiosin, during secondary metabolism [10] Prodigiosin has been reported to display antimalarial, antibacterial, antifungal, antiprotozoal, antitumour and immunosuppressant activities [62]. This is the first report that *S. liquefaciens* and *S. proteamaculans* isolated from bat guano are showing clear and distinct antifungal activity.

Overall, our data provide new information about the presence of *Serratia proteamaculans* found in bat guano from a subterrestrial aquatic ecosystem. This study shows that a small number of bacteria cells are able to have a highly competitive ability through the compounds that defuse into the agar against plant pathogenic fungi. Our study also provides novel data that *S. proteamaculans* and *S. liquefaciens* isolates are capable of prodigiosin production and new insights into the relationship between prodigiosin and other secondary metabolites as promising agriculture fungicides.

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THE ADAPTIVE POTENTIAL OF THE *Rosa canina* L. ROOTSTOCK OBTAINED in vitro IN THE CONDITIONS OF THE SOUTH OF WESTERN SIBERIA

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Abstract

Garden roses are reproduced by grafting cultivars on resistant rootstocks, predominantly intraspecific forms of *Rosa canina* L. The underground part of these rootstocks is winter-hardy even in the forest-steppe and southern taiga of Western Siberia. However, their shoot systems of formation (SSF), on which generative shoots are formed, related to the type of shortened fruit, are damaged during the wintering period. After extreme wintering, *R. canina* plants of ontogenetic states g1-g3 for one year can pass into the state of "temporarily non-flowering". In the Central Siberian Botanical Garden of the SB RAS (CSBG, Novosibirsk), long-term studies of seasonal development, ontogenesis, biomorphology, reproductive biology and winter hardiness of wild rose species from the *Caninae* Crėp sections are carried out. For the first time, the results of a long-term study of winter hardiness and seed productivity of the selected form of *R. canina* were presented, and an assessment of the SSF after extreme wintering, characterized by various damaging factors, were carried out. The aims of the present study were to establish in vitro conditions and propagate through direct organogenesis a promising winter-hardy form of *R. canina* used as a rootstock for garden roses, as well as to assess the adaptive potential of plants obtained by micropropagation under continental climate. In vitro experiments were carried out on a winter-hardy form selected in the CSBG from F_3 plants of local reproduction. The primary explants were meristems with two leaf primordia isolated from axillary buds of annual vegetative shoots. At the establishment stage the explants were cultured on hormone-free modified liquid Murashige and Skoog's medium (MS) supplemented with 100.0 mg/l glutathione and 30.0 g/l glucose. Direct organogenesis was induced on MS medium supplemented with 2.0 mg/l 6-benzylaminopurine (BAP) and 1.0 mg/l 3-indoleacetic acid (IAA). MS medium with 1.0 mg/l BAP was used for shoot multiplication. A hormone-free half-strength MS medium supplemented with 1.0 mg/l IAA was used to root the obtained microshoots. The regenerants were grown in containers with sterile sand, then in pots with a substrate consisting of a mixture of peat with perlite, humus, sand, and coconut substrate (1:1:0.5:0.5) and transferred to soil culture. Further studies were carried out in 2015-2021 on the experimental plot of the CSBG, located in the forest-steppe zone of the south of Western Siberia (Novosibirsk, Akademgorodok), which is characterized by a continental climate. In the study of morphogenesis, classical and modern biomorphological approaches were used, which consider the shrub form as a complex shoot systems of formation in space and the change of these systems in time. The seasonal dynamics of starch in the shoots was studied using a reaction with iodine in potassium iodide. Seed productivity was determined. At the multiplication stage, 8±1 microshoots per explant on MS media with 1.0 mg/l BAP was obtained. The in vitro rooting frequency was 60.0 % with a mean number of 2.0±1.0 roots per microshoot on half-strength MS with 1.0 mg/l IAA. It was revealed that the pregenerative period in vitro-derived *R. canina* plants is reduced by a year, compared with ones of seed origin. Plants enter the ontogenetic state g1 in the third year, and the formation of partial bushes, which can be used to seed plantations, begins in the fourth year of vegetation. Under the conditions of the continental climate of the forest-steppe of Western Siberia, all in vitro-derived specimens of the selected form had annual fruiting on shoots above the snow cover. The exception was 2020-2021, however, even after a severe wintering, hypanthia formed in the lower part of the SSF. A prolonged decrease in temperature to –30 °C in December led to partial damage to the middle part of the SSF, while a short-term decrease in air temperature to –28 °C in January did not cause serious damage even to the middle part of the SSF. The selected form was also resistant to strong spring frosts in the second decade of May. With favorable wintering and the preservation of terminal buds, the SSF of mature generative plants (g2) continued to increase in height. The combination of two favorable wintering periods in a series led to the formation and preservation of powerful SSF up to 2 m tall, as well as to the formation of mainly 2-3 hypanthia with a high number of completed seeds on short fruit shoots. Starch hydrolysis in those shoots of *R. canina*, in which complete leaf fall was observed in October (phenophase L5), was almost completed in November. However, single starch grains were still in single-row and multi-row medullary rays, as well as in the perimedullary zone.

Keywords: *Rosa canina*, clonal micropropagation, ontogenesis, partial bush, seed productivity, histochemical studies, forest-steppe of Western Siberia

Garden roses are traditionally grown in grafted culture [1-4]. In Siberia, there are no natural habitats for the most widely used rootstock *Rosa canina* L. which grows in Europe, Western and Central Asia, North Africa, the Crimea and the Caucasus. The eastern border of the range of this species does not reach the Kama [5].

The main problem is that the underground part of the *R. canina* rootstock has a high winter hardiness, while the aboveground shoot system, even in the forest-steppe of Western Siberia, often receives serious damage because of wintering. It is possible to successfully grow *R. canina* under-howling seedlings from seeds collected in natural habitats with a milder climate, but the production of seeds of local reproduction on an industrial scale in the region is not guaranteed.

Repeated attempts to use wild roses of the local flora (R. acicularis Lindl., R. majalis Herrm.) as rootstocks in the Urals and Western Siberia, as well as the most winter-hardy introduced wild roses (*R. rugosa* Thunb.) have shown that these species are unpromising. All of them are characterized by the presence of a large number of thorns and small spines on the shoots, which make grafting and budding extremely difficult, as well as high overgrowth, which further inhibits the varietal graft. *R. acicularis* and *R. majalis* also have a short period of good bark separation associated with sap flow and thin, bursting bark in the grafting zone [6].

According to foreign taxonomic and molecular genetic studies [7-9], *R. canina* is an extremely polymorphic species with a wide range, which allows the search and selection of ex situ forms that are promising in specific ecological and geographical conditions both as a rootstock and and as a medicinal plant [10-12]. Outside the natural range, in conditions of a more comfortable than continental monsoon climate of the Russian Far East, cases of naturalization of the introducent R. canina were noted [13].

Long-term studies of seasonal development, ontogeny, biomorphology, reproductive biology and winter hardiness of wild rose species from the sections *Caninae* Crėp., *Indicae* Thory, *Synstylae* DC have been carried out in the Central Siberian Botanical Garden of the Siberian Branch RAS (CSBG, Novosibirsk). and *Cinnamomeae* DC. Due to the special type of *Caninae* meiosis [14], as well as the tendency of *R. canina* and *R. corymbifera* to autogamy and facultative apomixis [15], the seed progeny of selected forms of these species is characterized by matroclinity. The most effective way to solve the problem of accelerated reproduction of winter-hardy highly productive forms is by using clonal micropropagation. Foreign biotechnologists described a positive experience of in vitro propagation of some species [16-18] and cultivars [19-21] of roses.

In this paper, for the first time, the results of a long-term study of winter hardiness and seed productivity of a selective highly winter-hardy form of *Rosa* *canina* propagated in vitro are presented, and an assessment is made of the state of the formation shoot system after extreme wintering. It was revealed that after extreme wintering plants of the selected form do not pass into the category of "temporarily not blooming".

The aim of the work was to assess the adaptive potential of a promising winter-hardy form of *Rosa canina*, introduced into culture in vitro.

Materials and methods. Reproduction of the winter-hardy form of *R. canina*, used as a rootstock for garden roses, was carried out using microcloning.

Plants of the initial introduction populations of *R. canina* were grown from seeds collected in natural habitats in Kabardino-Balkaria. First, the selection of the best two-year-old individuals of seed origin was carried out according to the growth energy in the pregenerative period, winter hardiness, moderate formation of renewal shoots, and resistance to fungal diseases. Further, samples with the highest yield of hypanthium fruits and regular fruiting were noted. The selected winter-hardy forms (in particular, *R. canina* no. 23 and *R. canina* no. 39) were used for comparison during further selections. To analyze the rhythms of growth and development, the degree of readiness of *R. canina* plants for wintering, the species of the local flora *R. majalis* Herrm was also involved in comparative studies.

Experiments in vitro were carried out on a selected winter-hardy form isolated in CSBG from F_3 plants of local reproduction. The primary explants were meristems with two leaf primordia isolated from axillary buds of annual vegetative shoots. At the stage of introduction into in vitro culture, the explants were cultured for 3 days in a liquid nutrient medium according to the Murashige-Skoog (MS) prescription, supplemented with 100.0 mg/l glutathione and 30.0 g/l glucose [22, 23]. To induce direct organogenesis, MS medium with 2.0 mg/l 6-benzylaminopurine (6-BAP) and 1.0 mg/l 3-indoleacetic acid (IAA) was used. Actually micropropagation of regenerants was carried out on MS medium with 1.0 mg/l BAP. Microplants were rooted on MS medium with half the content of micro- and macroelements, supplemented with 1.0 mg/l IAA. The transfer of regenerants to soil culture was preceded by cultivation in containers with sterile sand, then in pots with a substrate consisting of a mixture of peat with perlite, humus, sand and coco substrate (1:1:0.5:0.5) [24].

Further studies were carried out in 2015-2021 at the CSBG experimental site located in the forest-steppe zone of the south of Western Siberia (Novosibirsk, Akademgorodok) which is characterized by a continental climate. The meteorological conditions of vegetation and wintering were analyzed on the basis of data from the GMS closest to the CSBG (Ogurtsovo settlement, Novosibirsk).

Plants from pots with a substrate were transplanted into open ground. Phenological observations were carried out according to the described method [25].

When studying morphogenesis, classical and modern biomorphological approaches were used [26, 27], which consider the shrub form of growth as a set of shoot systems of formation (SSF) in space and the change of these systems in time. In the above-ground part of the bush, tillering shoots, stem and rhizome, were isolated, in the underground part, the xylopodia and xylorizome [28]. The dynamics of shoot formation was evaluated on 15 plants. Qualitative features of ontogenetic states were described on the basis of the ontogeny periodization scale [29].

The seasonal dynamics of the starch content in shoots was studied using a reaction with iodine in potassium iodide. The composition of the reagent was as follows: 2 g of potassium iodide, 0.2 g of crystalline iodine and 100 ml of distilled water [30]. Micropreparations were prepared using an MC-2 sledge microtome

(Spectro Lab, Ukraine) with a TOC-II thermal cooling table (Tochmedpribor, Ukraine), a Carl Zeiss Axioscop-40 light microscope (Carl Zeiss, Germany), and an AxioCam MRc-5 video camera (Carl Zeiss, Germany) with AxioVision 4.8 image acquisition and processing software (Carl Zeiss, Germany, https://carl-zeiss-axiovision-rel.software.informer.com). With regard to seed productivity, methodological guidelines for seed breeding of introducers [31] and our own developments [32] were used. Seed productivity in the studied forms of *R. canina* was assessed by 20 hypanthia fruits.

Statistical processing was carried out according to B.A. Dospekhov [33] in Microsoft Excel 2003. Formulas were used to calculate the statistical characteristics of the sample with quantitative variability of traits: arithmetic means (*M*), mean errors (±SEM), and coefficients of variation (*Cv*) were calculated.

Results. The features of morphogenesis of the selective winter-hardy form of *R. canina* which is characterized by a technologically valuable small number of thorns on shoots (Fig. 1, a) were studied in vitro in buds taken in September-October, since they had the maximum frequency of shoot formation (62.0 %).

Combination of 2.0 mg/l 6-BAP and 1.0 mg/l IAA with glutathione in the zero passage provided obtaining viable microshoots by the end of the first passage (after 6 weeks of culture). Regeneration of microshoots from the meristems of axillary buds occurred along the periphery of their base (see Fig. 1, b). On average, approx. three shoots per explant were formed. At the stage of actual micropropagation on media with 1.0 mg/L 6-BAP, the number of microshoots per explant was 8±1 (see Fig. 1, c). When rooting regenerants on MS medium with half the content of micro- and macronutrients and 1.0 mg/l IAA with a rhizogenesis frequency of 60%, each microshoot produced an average of 2 1 roots (see Fig. 1, d, e).

The survival rate of regenerated plants ex vitro was 95-100%, the plants were characterized by good developed aboveground part and root system, e.g., 2-4 roots of 2.5-7.5 cm in length (see Fig. 1, f, g). Plants with such morphological characteristics (see Fig. 1, h) were studied for their ontomorphogenesis and reproductive biology in vivo.

Initially, during a short time, a highly winter-hardy form was propagated in the amount necessary to create an experimental seed plantation with minimal use of shoot material. The pregenerative period in *R. canina* of microclonal origin was reduced by one year, individuals entered the ontogenetic state g1 in the third year, and the formation of partial bushes that can be used to replenish seed plantations began in the fourth year of vegetation. With seed propagation, it would take another 1-2 years to stratify the seeds.

The second stage of work was necessary since the meteorological conditions and damaging factors during almost half-yearly wintering in the conditions of the forest-steppe of Western Siberia vary greatly from year to year. This zone is characterized by a sharp decrease in temperature in November to –20... –25 °C, prolonged December and January frosts below –35 °C, insufficient accumulation of solid precipitation in the first half of wintering. Long-term studies carried out by us made it possible to cover all the problematic wintering periods and form the most complete picture of the adaptive potential of the selective winter-hardy form of *R. canina*. Particular attention was paid to the development and preservation of shoot systems after wintering, depending on meteorological conditions. In addition, hydrothermal conditions from the 2nd decade of September to the 3rd decade of October were important to achieve the end of the linear growth of the shoots and the initiation of the terminal bud.

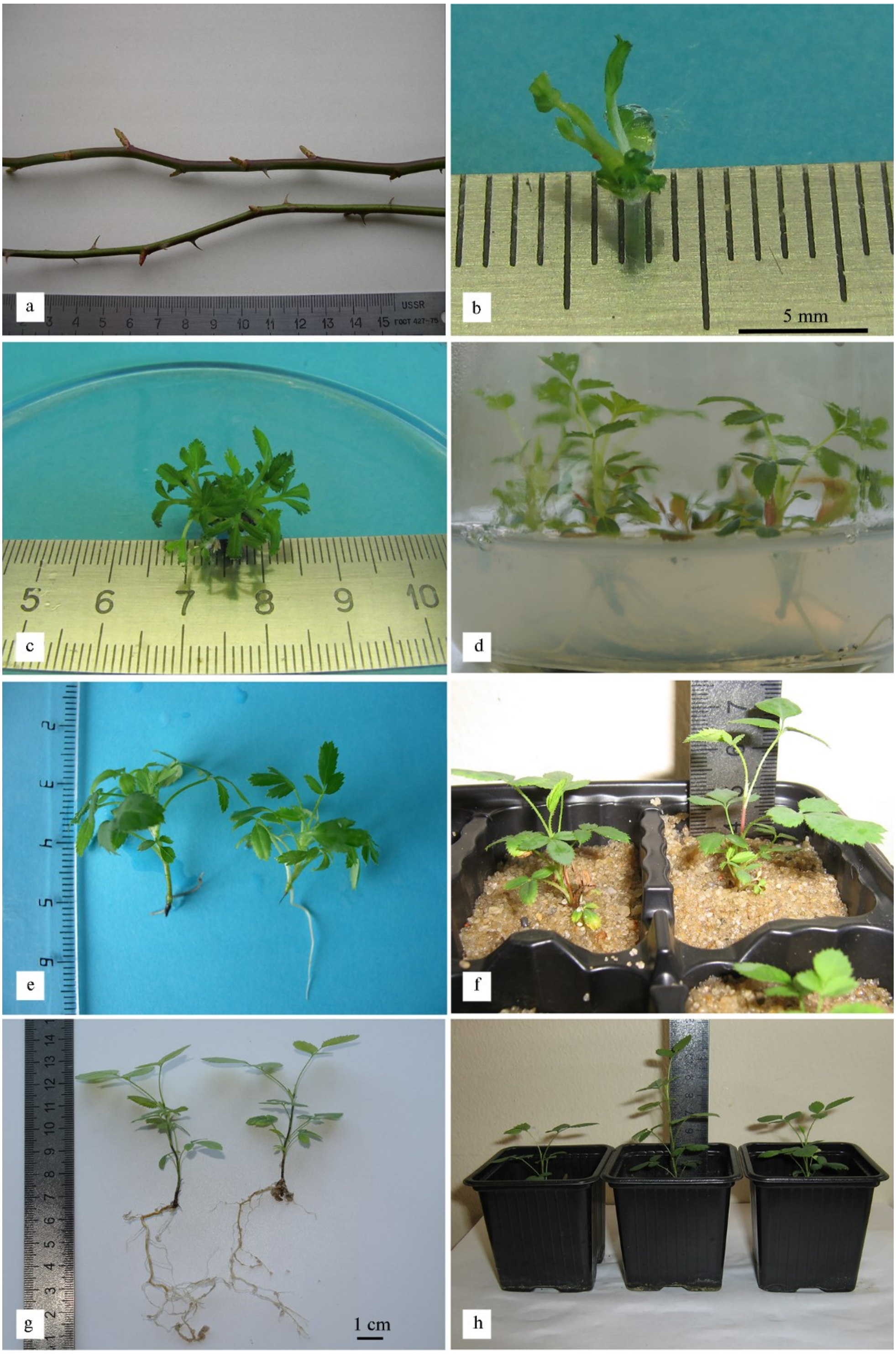

Fig. 1. Clonal micropropagation of the selected winter-hardy form *Rosa canina* L.: a — annual vegetative shoots, starting material for introduction into in vitro culture, b — formation of microshoots from the meristem with 2,.0 mg/l of 6-benzylaminopurine (6-BAP) and 1.0 mg/l of 3-indolylacetic acid, c — microshoots after 6 weeks of micropropagation on MS medium with 1.0 mg/l of 6-BAP, d — rooting of regenerants on medium 1/2 MS with 1.0 mg/l IAA, d — micro-plants after 6 weeks of cultivation on a rooting medium; sand and coco substrate (1:1:0.5:0.5) after 4-week adaptation.

1. Meteorological characteristics of the 2015-2021winter periods in the conditions of the continental climate of the forest-steppe of Western Siberia (Novosibirsk)

Month	Air temperature, °C								Precipitation, mm		
	decade						average per month	deviation from the norm	decade		
	I		II		III				I	II	III
	mean	min	mean	min	mean	min					
					2015						
November	–2.0	–10.8	–14.3	–21.1	–8.4	–24.9	–8.2	–0.6	6.0	0.0	30.0
December	–4.2	–15.1	–6.4	–17.7	–7.3	–27.1	–6.0	7.9	9.0	7.0	32.0
					2016						
January	–20.2	–29.3	–20.2	–34.8	–18.2	–29.7	–19.5	–2.7	1.0	5.0	0.0
February	–6.8	–19.6	–14.8	–26.3	–6.3	–20.3	–9.3	6.3	10.0	4.0	0.5
November	–6.1	–20.8	–22.3	–33.2	–10.2	–31.7	–12.9	–5.3	21.0	6.0	19.0
December	–10.4	–26.5	–9.4	–22.0	–16.6	–36.1	–12.1	1.8	23.0	19.0	11.0
					2017						
January	–8.9	–29.2	–19.2	–29.5	–15.1	–31.9	–14.4	2.4	13.0	6.0	5.0
February	–12.7	–21.0	–20.1	–32.8	–5.0	–16.4	–12.6	3.0	5.0	8.0	4.0
November	0.7	–6.9	–7.6	–22.9	–8.8	–24.8	–5.2	2.4	16.0	11.0	7.0
December	–9.3	–18.1	–17.9	–27.8	–9.7	–24.3	–12.3	1.6	5.0	1.0	33.0
					2018						
January	–20.2	–31.5	–15.7	–31.2	–26.8	–37.4	–20.9	–4.1	3.0	21.0	3.0
February	–14.9	–26.5	–15.4	–27.1	–15.5	–28.3	–15.3	0.3	0.8	3.0	1.0
November	–5.7	–22.5	–11.7	–23.0	–7.1	–17.1	–8.2	–0.6	27.0	16.0	27.0
December	–21.9	–36.5	–12.8	–28.1	–23.5	–35.4	–19.4	–5.5	6.0	11.0	5.0
					2019						
January	–15.5	–27.9	–13.8	–26.6	–14.7	–28.2	–14.7	2.1	3.0	2.0	7.0
February	–30.0	–40.1	–10.4	–25.8	–7.0	–14.7	–15.8	–0.2	0.0	4.0	4.0
November	–1.3	–14.8	–13.8	–29.5	–15.2	–28.5	–10.1	–2.5	14.0	14.0	8.0
December	–6.1	–22.5	–8.2	–13.9	–13.6	–31.6	–9.3	4.6	14.0	14.0	28.0
					2020 год						
January	–7.9	–22.4	–11.9	–25.8	–11.7	–28.8	–10.5	6.3	11.0	16.0	17.0
February	–7.9	–27.4	–10.8	–29.8	–4.9	–17.2	–7.9	7.7	19.0	7.0	9.0
November	2.2	–2.8	–7.0	–20.8	–9.3	–19.4	–4.7	2.9	11.0	18.0	0.2
December	–16.2	–25.3	–10.5	–21.5	–21.4	–40.0	–16.0	–2.1	10.0	10.0	20.0
					2021						
January	–27.2	–39.3	–19.0	–32.1	–19.1	–41.0	–21.8	–5.0	3.0	6.0	19.0
February	–11.2	–26.3	–16.4	–36.1	–21.5	–32.6	–16.4	–0.8	13.0	12.0	6.0

As our studies have shown, to more accurately predict the degree of SSF damage, it is necessary to take into account not only the average ten-day air temperature and the total amount of solid precipitation per decade, but also the minimum ten-day temperature (Table 1). Thus, the average air temperature in the third decade of November 2016 was –10.2 °C, and the minimum dropped to –31.7 °C. This beginning of wintering is considered extreme, since during the entire second decade of November the minimum air temperatures were below –20 °C, and during 6 days they even dropped to –25 °C. The deviation of the average monthly air temperature from the norm was 5.3 °C. The end of the growing season in 2016 with a sharp drop in temperature in October also did not contribute to the completion of the growth of annual shoots and the establishment of terminal buds.

2. The structure of a bush of young (g1) **and mature** (g2) **generative plants of the selected winter-hardy form of *Rosa canina* L. derived from clonal micropropagation in the conditions of the continental climate of the forest-steppe of Western Siberia** (n = 15, M±SEM; Novosibirsk, Akademgorodok)

Year, status	SSF		Bushing shoots		Stem shoots		Partial bushes	
	number	height, cm	number	height, cm	number	height, cm	number	height, cm
2016, g1	2.13±0.19	128.73±1.52	2.07±0.21	122.60±3.75	0		0	
2017, g2	3.47±0.13	143.13±1.83	2.60±0.67	155.80±2.88	7.33±0.43	45.93±1.42	0.67±0.13	92.80±2.23
2018, g2	5.33±0.32	175.93±3.32	4.13±0.26	159.27±1.12	5.87±0.24	23.33±2.18	2.13±0.09	96.20±1.05
2019, g2	2.13±0.09	119.00±1.50	2.13±0.09	119.00±1.50	2.13±0.09	119.00±1.50	2.13±0.09	119.00±1.50

N o t e. SSF — shoot system of formation.

After extreme wintering, young generative (g1) plants of the selected form did not go into the state of “temporarily not blooming”, although the SSF was damaged in the upper and partially middle parts of the shoots. The fruits were formed in the lower and middle tiers of the SSF.

Intensive formation of stem shoots during the growing season of 2017 (Table 2) was the result of the death of the upper and damage to the middle parts of the axial shoot due to extreme wintering. The most favorable beginning of the winter period was observed in November 2017 and November 2018.

The combination of two favorable winterings in a row led to the formation and preservation of powerful SSF up to 2 m tall, as well as to the formation of mainly 2-3 hypanthia with a high number of completed seeds on short fruit shoots (Table 3). In nursery, rosehip nuts, which are formed inside the hypanthium, are traditionally called seeds, and the hypanthium itself (overgrown receptacle) is called the fruit. In order to predict the yield of high-quality rootstock seeds from the harvested fruits, we counted the completed seeds and then correlated these indicators with the yield of harvested fruits in various selected forms, including the most winter-hardy.

3. Seed productivity of the selected winter-hardy form of *Rosa canina* L. derived from clonal micropropagation in the conditions of the continental climate of the forest-steppe of Western Siberia (*n* = 15; Novosibirsk, Akademgorodok)

Plant age, years	Seeds per fruit				Weight of filled seeds per fruit, g	
	filled		unfilled			
	M±SEM	*Cv*, %	*M*±SEM	*Cv*, %	*M*±SEM	*Cv*, %
			R. canina № 23			
4	36.32±0.90	24.3	6.35±0.45	34.2	0.82±0.02	14.3
5	29.72±1.05	35.6	11.40±0.62	42.0	0.86±0.02	11.7
6	32.95±1.16	22.1	7.82±0.56	39.4	0.95±0.02	9.8
			R. canina № 39			
4	38.05±1.23	29.7	5.42±0.40	44.5	0.88±0.03	12.0
5	35.21±1.37	19.2	8.10±0.63	32.1	0.94±0.02	8.4
6	32.71±0.90	18.6	7.30±0.47	37.3	0.97±0.03	9.3
			R. canina, selected form			
4	31.75±1.43	20.5	4.90±0.43	39.6	0.69±0.03	20.3
5	35.30±1.48	18.7	5.20±0.49	42.5	0.84±0.04	21.4
6	34.85±0.95	12.2	5.75±0.44	34.3	0.91±0.04	19.8

In the winter-hardy form, with favorable wintering and the preservation of terminal buds, the SSF of mature generative plants (g2) continued to increase in height. Long-term studies have shown that the SSF of the selected form of *R. canina* were the most unstable to low negative temperatures in November, when the process of transition to the state of winter dormancy has not yet been completed.

Histochemical express diagnostics of the readiness of wild roses for wintering was carried out taking into account the fact that by the beginning of leaf fall, the tissues of the shoots contain the autumn maximum of starch. In October-November, in winter-hardy species and forms, starch hydrolysis occurs most completely, and in tissues, this has a centripetal character. There are a single-row and multi-row medullary rays, perimedullary zone and core, but is already absent in the cortical parenchyma, phloem, cambium and xylem [6].

In those shoots of *R. canina*, in which complete leaf fall was observed in October, starch hydrolysis was almost completed in November, single starch grains were still in single-row and multi-row medullary rays, as well as in the perimedullary zone (Fig. 2, a) . In the highly winter-hardy local species *R. majalis*, which is used in studies as a control, single starch grains were found only in the perimedullary zone (see Fig. 2, b). Consequently, in both species, the content of starch in the tissues was minimal and was estimated at 1 point. Histochemical studies of *R. canina* shoots in the pre-winter period [6, 34] carried out at the Central Siberian Botanical Gardens revealed differences in starch dynamics even among fairly winter-hardy forms: starch grains in single-row and multi-row medullary rays could be located in less than 50% of the cells, which corresponds to 2 points.

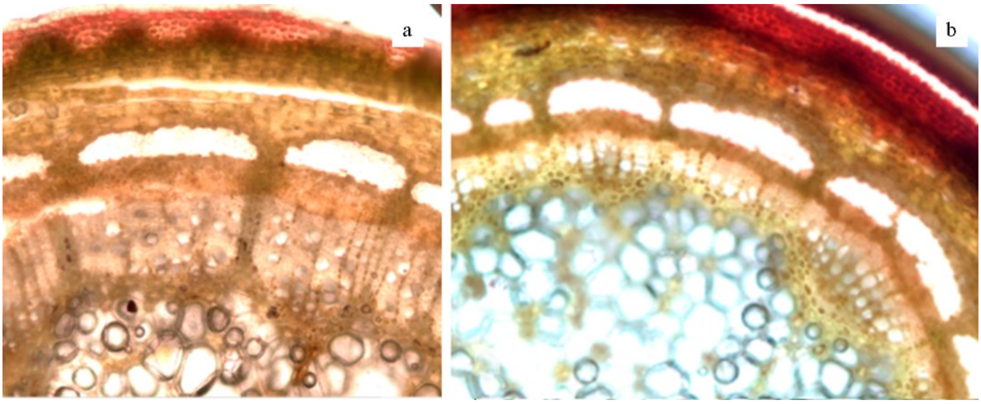

Fig. 2. The content of starch in the tissues of annual shoots of *Rosa canina* L. (a) **and *R. majalis* Herrm.** (b) **in the pre-winter state** (Novosibirsk, Akademgorodok, November 2021; iodine—potassium iodide syaining, magnification ×40, light microscope Carl Zeiss Axioscop-40, Carl Zeiss, Germany; video camera AxioCam MRc-5, Carl Zeiss, Germany, with AxioVision 4.8 imaging software, Carl Zeiss, Germany, https://carl-zeiss-axiovision-rel.software.informer.com).

A prolonged (for more than 2 days) decrease in temperature to –30 °C in December led to partial damage to the middle part of the SSF. A short-term (for several hours during the day) decrease in temperature ranged from –25 to –28 °C in January did not cause serious damage even to the middle part of the SSF (for example, in the winter of 2018-2019).

Wintering of 2020-2021 was also among the extreme ones, during which in the third decade of December, as well as in the first and third decades of January, the minimum temperature dropped to –41 °C. As a result, the upper and partially middle parts of the SSF were died. A negative impact on the development of the generative sphere of many fruit crops, as well as the death of flowers of grape varieties and actinidia at the beginning of the growing season of 2021 in the conditions of Novosibirsk, was also exerted by severe frosts on May 20 (–3.2 °C) and on May 21 (–6.0 °C). These days, the average daily temperature was 6.8 and 8.5 °C below the norm. Nevertheless, in the basal and middle parts of the SPF, on the short fruit shoots of the selected form of *R. canina*, predominantly single hypanthia were formed, which confirms the high winter hardiness and frost resistance (Fig. 3, a).

Seed material, as a rule, is intended for obtaining seedlings of wild rose rootstocks on an industrial scale, and it is promising to use partial bushes for the speedy expansion of mother plantations. The studied selective form was characterized by the accelerated formation of partial bushes (see Fig. 3, b), which served as one of the marker signs of the transition to the mature generative state (g2).

Creation of initial introduction populations of *R. canina* by two-stage selection made it possible to carry out further selection of winter-hardy highly productive forms among plants grown from seeds of local reproductions. The analysis of selected forms grown from seeds of the first local reproduction has proved the prospects of these research works. Comparison of the *R. canina* best forms in the Central Siberian Botanical Garden and in one of the nurseries of Holland [35] in terms of the yield per bush showed that individual forms of *R. canina* during the period of maximum fruiting under local conditions have the same high productivity as in Dutch highly specialized enterprise.

Fig. 3. Mature generative plants (g2) **of the *Rosa canina* L. selected form:** a — fruiting in the lower part of the formation shoot system after extreme wintering, b — partial bush (Novosibirsk, Akademgorodok, 2021).

An analysis of studies conducted with wild rose rootstocks abroad [1, 7, 10] showed that the problems of increasing the winter hardiness of rootstocks in a temperate continental and milder climate are irrelevant. The main selections are carried out for resistance to fungal diseases, and the issues of overcoming deep seed dormancy are also being addressed. Research carried out at the Central Siberian Botanical Garden and focused on the identification of winter-hardy forms of rootstocks of garden roses with high seed productivity are of great importance for regions with harsh climatic conditions and make it possible to avoid mass purchases of rootstock seedlings in the southern regions of Russia and the CIS. The features of the reproductive biology of wild roses revealed in the process of research [15] are of wider theoretical interest. From an ecological point of view, the assessment of adaptive potential will also be of importance, carried out in many respects, in particular, using histochemical studies using modern instrumentation with digital image processing.

Thus, the maximum frequency of shoot formation (62.0%) in the selective winter-hardy form *Rosa canina* was noted in buds taken in September-October. It was revealed that the pregenerative period in *R. canina* of microclonal origin is reduced by a year compared to plants of seed origin, therefore, individuals enter the ontogenetic state g1 (young generative) in the third year of vegetation. Partial shrubs that can be used to replenish seed plantations are formed in the fourth year of the growing season. Under the conditions of the continental climate of the forest-steppe of Western Siberia, in all specimens of the selected form, propagated in vitro, annual fruiting was noted on shoots above the snow cover. The exception was the wintering of 2020-2021, however, even after a severe wintering, hypanthia formed in the lower part of the shoot systems of the formation. An important feature of the phenorhythmics of the selective form of *R. canina* is the ability to complete growth and lay a terminal bud on most shoots of the current year, that is, to fully prepare for wintering, which is confirmed by histochemical studies of the dynamics of starch content.

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SIMULATION OF VEGETATION CONDITIONS USING DIFFERENCES OF CURRENT NDVI VALUES FROM AVERAGE LONG-TERM INDICATORS

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Abstract

Currently, one of the important tools for increasing crop production is the introduction of precision farming systems. As an obligatory element of such systems, production process control has been successfully used in recent years. Such control is implemented by modeling the responsiveness of the vegetative mass to changes in actual environmental conditions. In domestic and foreign literature, there are many examples of the development of mathematical models of plant growth and development that take into account external influences. It is shown that the predictive models allow us to respond in a timely manner to changing growing conditions. In turn, this helps to quickly make optimal agronomic decisions. In this work, for the first time, the relationship between the difference (anomaly) of the average annual and current seasonal indicators of NDVI (normalized difference vegetation index) and the process of plant growth and development, taking into account the influence of existing conditions, was established for the first time. It is shown that the conditions for the adequacy of approximation, when leveling noisy time series, are completely satisfied by the Gauss-Laplace function. As a mathematical expectation, the average values of the highest NDVI values of the vegetative period of the crop should be used. Mathematical models of the influence of photosynthetic, meteorological, and soil-climatic factors on NDVI anomalies in a particular phase of plant development have been obtained. Our goal was to develop predictive models of the vegetation process of grain crops, based on a comparison of the average long-term indicators of NDVI with its current seasonal values. The influence of actual conditions was taken into account. The research was carried out on the fields of the «Integration» center of the Oryol State Agrarian University (Oryol Procince). In 2021, winter wheat (*Triticum aestivum* L.) cultivar Moskovskaya 39 occupied an area of 48.1 ha, spring barley (*Hordeum vulgare* L. sensu lato) cultivar Raushan — 17.4 ha. Data for calculation of NDVI values were obtained from the CosmosAgro geoportal, as well as using an Agrofly Quadro 4/17 unmanned aerial vehicle (Agrofly International, Russia). Data noise compensation was performed by approximating time series with the Gauss-Laplace function. The adequacy of the regression models for the approximation of NDVI time series was assessed using the Fisher *F*-test and the average error of the approximation coefficient; the accuracy of the predictive models was confirmed by the Mean Absolute Percentage Error (MAPE) indicator. As a result, time series of the average NDVI value for the studied crops were obtained based on long-term observations, and the current NDVI values in the growing season 2021 were calculated. The distribution of time series of the vegetation index has been established. It was close to normal. The maximum (peak) values of NDVI are determined. They amounted to 0.71 for winter wheat and 0.54 for spring barley and fell in June, regardless of the crop. The purpose of leveling the noisy NDVI time series of crops during the growing season is most fully satisfied by the asymmetric Gauss-Laplace function. As a mathematical expectation, the average value of the highest NDVIs for the crop vegetation period was used. Mathematical models were obtained based on the NDVI anomaly index. These models describe the influence of photosynthetic, meteorological, soil, and climatic factors on the crop state during a particular phenophase. The mean absolute error of the proposed models was 9.23 for spring barley and 5.68 for winter wheat. Thus, the proposed characteristic ΔNDVI can be used as an independent variable (optimization criterion) in factorial models for predicting the dynamics of the vegetation process.

Keywords: yield forecast, vegetation index, NDVI, Gaussian function, factor analysis, time series approximation

Agriculture is on the verge of a digital revolution, which becomes the basis for precision farming and contributes to the implementation of the innovative development strategy of the Russian Federation. Site-specific crop management (SSCM) is an important element of precision farming, which is actively implemented to increase crop yields [1-4]. Regulation of the bioproduction process is possible due to timely and prompt response to deviations caused by external influences [5-8]. The latter include the soil-climatic factor, various plant diseases, pests, and weeds.

As tools for assessing the impact of environmental conditions on agricultural crops, the analysis of meteorological data and the values of the vegetation index is successfully used. Taking into account external influences allows not only to quickly respond to emerging deviations [9, 11], but also to increase the efficiency of monitoring the phytosanitary condition of crops [12], create new software products for analyzing incoming information [13, 14], develop and implement automated systems decision-making on plant protection [15], contributing to an increase in the productivity of agrocenoses. The methods of mathematical statistics [16], in particular, multivariate analysis [17) make it possible to carry out forecasting for the management of the vegetation process.

Previously, it was shown [18] that taking into account the influence of air temperature, soil moisture, and ultraviolet radiation power on the timing of plant development makes it possible to predict the vegetation process and develop recommendations for agronomic measures. It should be noted that the performance of the proposed method of factor analysis is determined by the choice of a characteristic indicator of the solution of the problem, by the value of which the optimality of the found algorithm is estimated. The implementation of the factor complex, in which the optimization criterion was the period of lagging/advancing the development of plants from the average values calculated from long-term data, made it possible to characterize the course of the process under study, which fully satisfies the task of obtaining an adequate mathematical model, while the predicted harvesting period harvest allowed to reduce the seasonal load of combines. However, this does not allow the assessment to be carried out remotely, which could be used when managing a household based on digital platform solutions.

Thanks to the methods of remote sensing of the Earth (ERS), the amount of information received and the possibilities of its processing are expanding [19-21]. One of the indicators reflecting the assessment of the state and dynamics of plant development is the normalized difference vegetation index (NDVI). To predict the influence of existing conditions on the state of plants, it is advisable to use the method of comparing current values with long-term averages. At the same time, in order to exclude the features of a particular growing season (advance or lag in development), the averaged NDVI time series should be leveled [22-25]. This will make it possible to analyze information on deviations of current values from long-term averages at comparable stages of plant development with a smaller error [26].

In this work, for the first time, the relationship between the difference (anomaly) of the average annual and current seasonal indicators of the normalized vegetative index NDVI and the process of plant growth and development under the influence of existing conditions has been established. It is shown that the use of the average value of the highest NDVI indicators of the growing season of a crop as the mathematical expectation of the Gauss-Laplace function for leveling noisy time series fully satisfies the conditions for the adequacy of their approximation. Mathematical models of the influence of photosynthetic, meteorological and soil-climatic factors on NDVI anomalies during a particular phase of plant development were obtained.

Our goal was to create predictive models of the state of the vegetation process of grain crops under the influence of existing conditions based on a comparison of the average long-term indicators of the NDVI vegetation index with its current seasonal values.

Materials and methods. The research was carried out on the fields of the Scientific and Educational Production Center "Integration" of the Orlov State Agrarian University (Orel Province). In 2016-2020, the average long-term values of the NDVI index were calculated for winter wheat in plots No. 28 (2016), Nos. 23, 26, 31 (2017), No. 36 (2018), Nos. 22, 33 (2019 year), Nos. 23, 24, 26 (2020), for spring barley - in plots Nos. 27, 30 (2016), No. 54 (2017), Nos. 37-39 (2018), No. 27, 34 (2019), No. 13 (2020). In the growing season of 2021, experimental crops of winter wheat (*Triticum aestivum* L.) variety Moskovskaya 39 occupied an area of 48.1 hectares, spring barley (*Hordeum vulgare* L. sensu lato) variety Raushan 17.4 hectares.

Normalized difference vegetation index (NDVI) was calculated by the formula [27]:

$$NDVI = \frac{NIR - red}{NIR + red},$$

where *NIR* is the vegetation cover reflection in the near infrared region (0.85-0.88 μm) of the electromagnetic spectrum and *red* in the red region (0.64-0.67 μm).

Satellite data for 2016-2020 were obtained on the CosmosAgro geoportal developed by the ScanEx Engineering and Technology Center (Russia) [28]. We used multi-temporal archival remote sensing data from the Sentinel-2 imaging system (MSI scanner, multichannel), free from clouds (no more than 10%), haze and other adverse factors, with a spatial resolution of 10.2 m/pixel and the frequency of obtaining information once at 5 days For analytical processing, the ScanEx GeoMixer utility [29] was used.

To obtain data on NDVI during the growing season of 2021, an unmanned aerial vehicle Agrofly Quadro 4/17 (Agrofly International, Russia) was used. Compensation for data noise caused by cloudiness, haze, evapotranspiration, precipitation, and other natural-climatic and temperature influences was performed by the approximation method. We used the asymmetric Gauss-Laplace function, which most fully meets the tasks of aligning the NDVI time series during the growing season [30-32]:

$$f(x) = \frac{1}{\sigma\sqrt{2\pi}} e^{-\frac{1}{2}\left(\frac{x-\mu}{\sigma}\right)^2},$$

where σ^2 is distribution variance; μ is mathematical expectation (average value).

Long-term statistical data on the dynamics of changes in the vegetation index NDVI were obtained from archival materials on crops with similar crops located near the plots of the field experiment.

The fairness of using average NDVI values for individual fields to describe the average annual crop indicator was confirmed by the comparison criterion. At the same time, due to the impracticability of the classical conditions for applying the Student's *t*-test in most statistical problems, the assessment of the homogeneity of time series according to the NDVI index was performed using the Cramer-Welch test T for the equality of mathematical expectations based on statistics [33]:

$$T = \frac{\sqrt{mn}(\bar{x}-\bar{y})}{\sqrt{n\sigma_x^2+m\sigma_y^2}},$$

where m, n are sample sizes; $\bar{x}$, $\bar{y}$ are the mean sample values; σ_x^2, σ_y^2 are the variances of sample distributions.

By comparing the T-test with the boundary value $\Phi\left(1-\frac{\alpha}{2}\right)$ where α is a significance level equal to 0.05, a decision was made to accept the hypothesis of homogeneity of the compared samples at the significance level α in accordance with the equality:

$$T \le \Phi\left(1-\frac{\alpha}{2}\right).$$

The results of biometric calculations were processed in the Microsoft Excel software environment. The arithmetic mean values ($\bar{X}$), standard deviations (σ), coefficients of variation (k_V) and dispersion (σ^2) for the samples were calculated, artifacts were searched for and excluded, and the distribution parameters of the variation series were studied. The error in the calculated values did not exceed 5%.

The adequacy of the regression models for the approximation of the NDVI time series was assessed using the Fisher's F-test and the average error of the approximation coefficient ($\bar{A}$) according to the following formulas.

$$F = \frac{\sigma_x^2}{\sigma_y^2},$$

where σ_x^2, σ_y^2 are variances of compared regression series;

$$\bar{A} = \frac{1}{n}\sum_{i=1}^{n}\left|\frac{y_i-\hat{y}_i}{y_i}\right| \times 100\ \%,$$

where y_i, $\hat{y}_i$ are the actual and theoretical (calculated by the regression equation) values, respectively, of the effective trait.

The accuracy of the predictive models was evaluated using the Mean Absolute Percentage Error (MAPE) model [6] based on the data for each phase of plant development in the growing season of 2021. At the same time, the prediction error was determined by comparing the actual NDVI anomaly index (ΔNDVI) with its theoretical values found for each characteristic segment of the growing season [34]:

$$MAPE = \frac{1}{n}\sum_{i=1}^{n}\frac{|y_{itheor}-y_{iactual}|}{y_{iфакт}} \times 100,$$

where n is the number of compared pairs of values; $y_{i\text{theor}}$, $y_{i\text{actual}}$ are the values of the indicators of the mathematical model optimization criterion and the actual indicators of the feature obtained during the experiment

Results. Despite some deviations at the end of the growing season, obviously caused by different harvesting times, the calculation of the Cramer-Welch criterion did not reveal significant differences in the compared variation series of the NDVI index for individual plots located near fields with experimental crops in 2021: the calculated values of T did not exceed boundary value $\Phi\left(1-\frac{\alpha}{2}\right)$ at a significance level $\alpha = 0.05$ (Table 1). This confirms the validity of using the values of the vegetation index of the selected plots to calculate the average annual NDVI values.

The seasonal dynamics of the NDVI index change according to long-term data is presented in Table 2. As can be seen, the nature of the change in the values of the time series was similar for the studied crops and, more than other distribution functions, corresponded to the normal law. Regardless of the crop, the lowest values of the vegetation index corresponded to the winter months. The highest

NDVI values were observed between May and June. The maximum average long-term values of the vegetation index were in June and amounted to 0.71 for winter wheat and 0.54 for spring barley.

1. Evaluation of the homogeneity of time series according to the normalized difference vegetation index (NDVI) **during investigation** (Orel Province)

Test sites	NDVI statistical parameters			
	arithmetic mean, $\overline{X}$	dispersion, σ^2	Cramer-Welch test, T	boundary T-value, $\Phi\left(1-\frac{\alpha}{2}\right)$, $\alpha = 0.05$
		2016		
		Spring barley (*Hordeum vulgare* L. sensu lato)		
No 27	0.47	0.220	$0.45_{27, 30}$	1.96
No 30	0.45	0.210		
		2017		
		Winter wheat (*Triticum aestivum* L.)		
No 23	0.48	0.085	$0.23_{31, 26}$	1.96
No 26	0.39	0.082	$1.22_{26, 23}$	
No 31	0.40	0.052	$1.68_{23, 31}$	
		2018		
		Spring barley (*Hordeum vulgare* L. sensu lato)		
No 37	0.34	0.012	$0.11_{37, 39}$	1.96
No 38	0.32	0.011	$1.67_{37, 38}$	
No 39	0.35	0.016	$1.64_{38, 39}$	
		2019		
		Winter wheat (*Triticum aestivum* L.)		
No 22	0.37	0.160	$0.10_{22, 33}$	1.96
No 33	0.37	0.220		
		Spring barley (*Hordeum vulgare* L. sensu lato)		
No 27	0.31	0.024	$1.09_{27, 34}$	1.96
No 34	0.29	0.026		
		2020		
		Winter wheat (*Triticum aestivum* L.)		
No 23	0.44	0.201	$0.19_{23, 24}$	1.96
No 24	0.46	0.223	$1.74_{24, 26}$	
No 26	0.42	0.231	$0.51_{23, 26}$	

2. Monthly values of the normalized difference vegetation index (NDVI) **across a 5-year study study** (Orel Province)

Year	Month											
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
		Winter wheat (*Triticum aestivum* L.) cv. Moskovskaya 39										
2016	нд	–0,02	0,30	0,26	0,55	0,71	0,44	0,39	0,36	0,28	0,26	nd
2017	–0.03	–0.03	0.19	0.32	0.43	0.74	0.73	0.54	0.55	0.46	0.48	0.20
2018	нд	–0.03	0.35	0.16	0.53	0.70	0.36	0.40	0.39	0.29	0.17	nd
2019	–0.04	нд	0.26	0.29	0.58	0.65	0.39	0.26	0.23	0.20	0.34	nd
2020	нд	нд	0.42	0.42	0.76	0.79	0.39	0.32	0.38	0.35	0.19	0.21
Average	–0.04	–0.03	0.33	0.27	0.54	0.71	0.46	0.38	0.38	0.27	0.28	0.21
		Spring barley (*Hordeum vulgare* L. sensu lato) cv. Raushan										
2016	–0.04	нд	0.18	0.19	0.36	0.55	0.53	0.42	0.32	0.22	0.2	nd
2017	–0.04	–0.03	0.30	0.33	0.50	0.56	0.80	0.54	0.49	0.48	0.23	0.03
2018	нд	–0.04	нд	0.20	0.27	0.48	0.47	0.39	0.33	0.26	0.19	nd
2019	–0.03	нд	0.14	0.14	0.37	0.56	0.52	0.32	0.19	0.19	0.30	nd
2020	–0.01	нд	0.17	0.19	0.27	0.54	0.41	0.37	0.33	0.21	0.13	0.13
Average	–0.04	–0.03	0.19	0.19	0.35	0.54	0.53	0.41	0.33	0.24	0.22	0.11

Note. nd — no data.

We carried out a comparative assessment of the average annual indicators of the vegetation index of the studied crops for 2016-2019; 2020 was not considered due to a clear deviation in NDVI values for compared crops due to lack of rainfall. This anomaly, especially in the spring and early summer periods, predetermined a sharp decrease in the vegetative mass of spring barley. The latter, as is known [35, 36], is more susceptible to lack of moisture compared to winter crops, which make better use of the spring reserves of moisture and nutrients.

A stable ratio of NDVI values for winter wheat to those for spring barley was found, which was 1.16 (16%) on average over the years for the specified period. At the same time, the correlation coefficient (r) between the compared time series turned out to be 0.96.

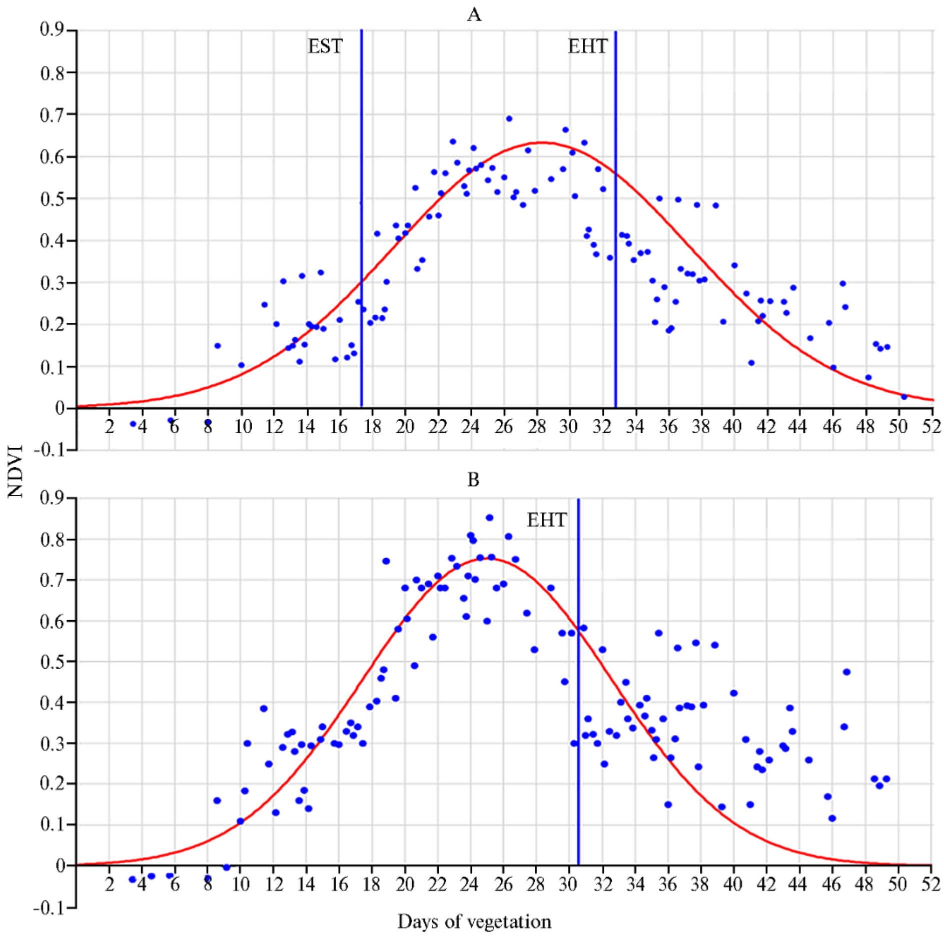

Fig. 1. The alignment of the average long-term time series of the normalized difference vegetation index (NDVI) **using the Gauss-Laplace function for spring barley (*Hordeum vulgare* L. sensu lato) cv. Raushan** (A) **and winter wheat (*Triticum aestivum* L.) cv. Moskovskaya 39** (B)**:** blue dots — actual values, graph — calculated values, EST — estimated sowing time, EHT — estimated harvest time (Orel Province).

Based on the use of the Gauss-Laplace function, an approximation of the actual NDVI time series was performed based on long-term average data and plots of regression models with a variable structure were plotted (Fig. 1). It is known [26] that one of the main conditions for the approximation of empirical series is the minimization of the sum of squared deviations of the theoretical points $\bar{y}_x'$ of the regression line from the points y_i of empirical (experimental) observations: $Q = \sum(y_i - \bar{y}_x')^2 \Rightarrow \min$. When using the Gauss-Laplace function, this requirement was provided by the values of the parameters σ and μ. Thus, the mathematical expectation μ was taken equal to the average value for the five highest NDVI indicators of the growing season for the crop. To equalize the time series of the vegetation index for winter wheat and spring barley, μ was 181 and 198, respectively. In both cases, the shift in the position of the mathematical expectation relative to the centers allows us to classify the obtained approximations as functions of an asymmetric left-hand distribution.

As can be seen, the description of the NDVI time series using the Gauss-

Laplace function made it possible to get rid of the noise caused by the difference in the conditions for obtaining the initial data.

Checking the adequacy of the accepted mathematical models using the Fisher's *F*-test and the average error of the approximation coefficient $\bar{A}$ in the areas characterizing the timing of the vegetation process of crops showed satisfactory convergence of the actual and theoretical series:

winter wheat — $F_{0.05}^{calc} = 1.20 < F_{0.05}^{test}(76) = 1.47$; $\bar{A} = 23.4\%$;

spring barley — $F_{0.05}^{calc} = 1.22 < F_{0.05}^{test}(50) = 1.6$; $\bar{A} = 19.9\%$.

A fairly high value of $\bar{A}$ was due to a large variation in the actual long-term average NDVI indicators (coefficients of variation $k_v = 0.50$ for winter wheat, $k_v = 0.51$ for spring barley). Nevertheless, based on the comparative assessment of the *F*-test, we believe that the result obtained gives the right to recommend these mathematical models for a comparative analysis of the deviations of the current values of the NDVI index of a crop from the long-term average data.

The average long-term values of the NDVI index for the studied crops differed somewhat from the dynamics in the growing season of 2021 (Fig. 2, A, B). In June 2021, NDVI values turned out to be higher; in July, they were lower than the average long-term observations. For both crops, the NDVI values were higher than the long-term average in the heading phase. Thus, with the maximum long-term average NDVI for winter wheat and spring barley of 0.75 and 0.63, respectively, the highest values of this indicator in 2021 for these crops were 0.80 and 0.74.

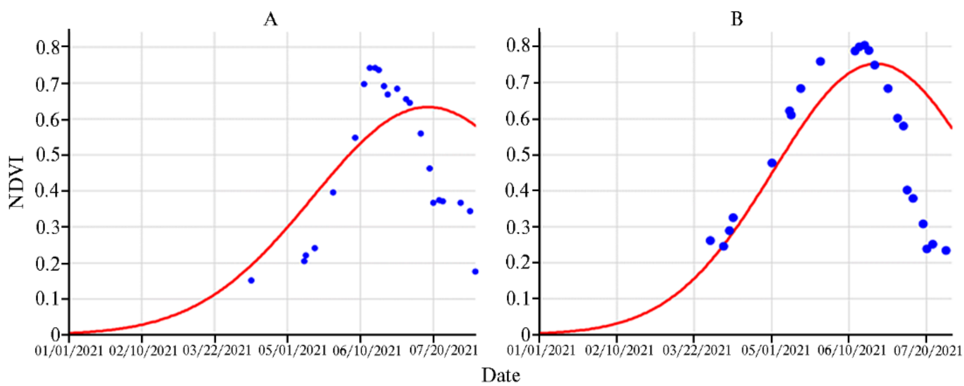

Fig. 2. Deviations of the normalized difference vegetation index (NDVI) **from the average long-term indicators in 2021 for spring barley (*Hordeum vulgare* L. sensu lato) cv. Raushan** (A) **and winter wheat (*Triticum aestivum* L.) cv. Moskovskaya 39** (B)**:** blue dots — actual values, graph — long-term averages (Orel Province).

The peak of the increase in NDVI in 2021 fell on June 15-20, which is 7-9 days earlier than the long-term average. Accordingly, an earlier decrease in the vegetation index associated with the completion of growth processes was observed compared to the average long-term norm. It was established that the optimal value of NDVI, equal to 0.30-0.35 and characterizing the readiness of the field for harvesting, was achieved for spring barley on August 11, for winter wheat on July 15. This is 2-2.5 weeks earlier than the average long-term deadlines for the end of the vegetation process. That is, for the growing season of 2021, we should state an advance relative to the long-term average normal values.

A diagram showing NDVI anomalies in 2021 (deviation of NDVI values from the average) (Fig. 3) can be used as a basis for assessing the influence of certain external factors on the change in the vegetation index. This will allow timely adjustment of agronomic measures, creating conditions favorable for the crop growth and developemt.

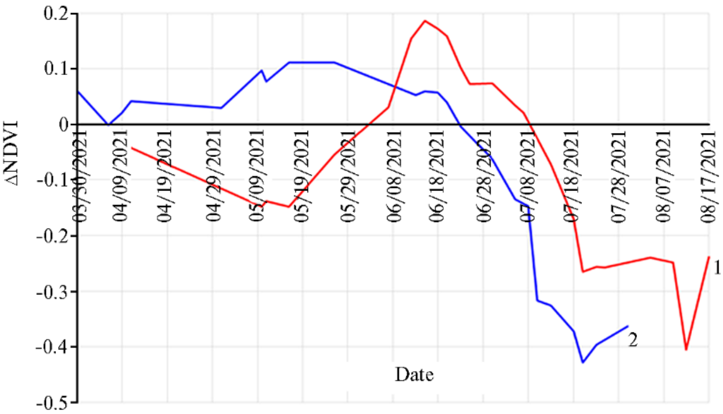

Fig. 3. Deviation of the the normalized difference vegetation index (ΔNDVI) **from the average long-term indicators during the growing season of 2021 for spring barley (*Hordeum vulgare* L. sensu lato) cv. Raushan** (1) **and winter wheat (*Triticum aestivum* L.) cv. Moskovskaya 39** (2) (Orel Province).

An assessment of the possibility of using deviations (anomalies) of the current values of the NDVI vegetation index from the long-term average made it possible to apply the previously obtained mathematical models that describe the influence of photosynthetic, meteorological, and soil-climatic factors on NDVI anomalies during a specific phase of plant development:

$$\Delta y_{sp} = -0.022 + 0.136x_2 - 0.184x_3 - 0.006x_5 - 0.002x_6 + 0.002x_8,$$

$$\Delta y_{wn} = -0.296 + 0.144x_1 + 0.004x_4 + 0,.021x_7 - 0.005x_8,$$

where x_1 is the content of chlorophyll a (mg · g^{-1}), x_2 is the content of chlorophylls a + b (mg · g^{-1}), x_3 is the content of carotenoids (mg · g^{-1}), x_4 is the soil temperature (T, °C), x_5 is the soil moisture (W, %), x_6 is the ambient air temperature (t, °C), x_7 is the accumulated amount of precipitation (ΣRN, mm), x_8 is the level of ultraviolet radiation (UV, W · m^{-2}).

Figure 4 shows the actual and modeled regression curves of the influence of acting factors on the NDVI anomalies of the 2021 growing season.

The assessment of accuracy by the MAPE indicator revealed a satisfactory average absolute error of the models: for spring barley 9.23, for winter wheat 5.68. Some decrease in the estimate of the accuracy of the predictive model for spring barley was probably due to the greater variability in seasonal NDVI values. So, if the variance of the vegetation index series for barley was 0.026, then for winter wheat it was 0.016. However, in general, the accuracy of the proposed models allows us to recommend them for practical use in production conditions. Regular assessment of current anomalies (for example, before the onset of the next crop phenophase and especially during the earing period) provides a real opportunity for operational management of the vegetation process to form maximum yields under specific conditions.

The results of numerous studies [37-39] demonstrate the practical applicability of the indicators of the normalized difference vegetation index for predicting the yield of cereals and other crops. At the same time, there is a higher correlation between the actual productivity of crops and the maximum (peak) NDVI values during the beginning of the heading phase [40, 41]. Some reports [42-44] discuss in detail the possibilities of using predictive models to assess the state of the vegetative mass, as a tool for managing the production process. Particular attention is paid to the processes of formation of yield, growth of the root system, changes in the composition of dry matter in plants, etc. This takes into account photosynthesis, respiration, transpiration and soil hydraulics, autotrophic processes and stomatal control. The results of studies are given for a number of crops - corn [41], cotton [43], soybeans [37], sugar beets and potatoes [38], forage grasses [40]. However, the characteristics of the relationship between the vegetation process and the dynamics of NDVI during individual phenophases are not studied, and attention is not focused on the influence of weather and climate impacts.

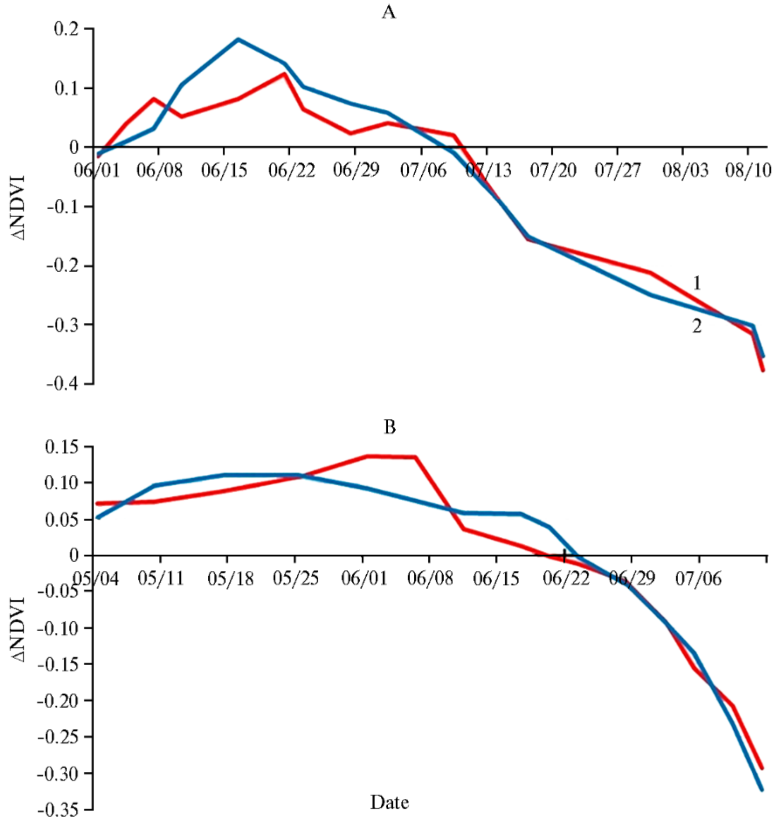

Fig. 4. Actual and simulated deviations of normalized difference vegetation index (ΔNDVI) **from the average long-term values during the growing season of 2021 for spring barley (*Hordeum vulgare* L. sensu lato) cv. Raushan** (A) **and winter wheat (*Triticum aestivum* L.) cv. Moskovskaya 39** (B): **1 — calculated anomaly 2 — actual anomaly** (Orel Province).

The approach proposed in this paper to the construction of a predictive model of the growing conditions of grain crops shares the goals formulated in the above works, but adds new aspects to them. A qualitative indicator of the process of growth and development of plants is a comparative assessment of the vegetation index, calculated from the results of the average annual and current seasonal values of NDVI. A short-term forecast of the state of plants is built on the basis of operational information about external influences. This approach is very important for a timely and reliable assessment of the current conditions and making an adequate decision on agrotechnical measures. In addition, unlike the known models with a daily step, the new model is based on the use of a complex indicator that takes into account the input parameters observed in real time. In addition to NDVI indicators, these are atmospheric and soil-climatic characteristics. In practice, the use of the proposed forecast algorithm and the corresponding set of monitoring tools will allow you to quickly respond to changing external influences and make the right agronomic decisions.

Thus, the task of managing the vegetation process of agricultural crops can be implemented on the basis of predictive models obtained through factor analysis of influencing external conditions. We considered the possibility of using deviations (anomalies) of the current seasonal values of the NDVI vegetation index from the long-term average as a dependent variable for a multivariate regression model. The purpose of leveling the noisy time series of NDVI of agricultural crops during the growing season is most fully satisfied by the asymmetric Gauss-Laplace function, where the average value of the highest NDVI indicators of the crop growing season is used as a mathematical expectation. As a result of a comparative analysis of the long-term average and the current (vegetation season 2021) NDVI indices for the studied crops, a diagram of NDVI anomalies (ΔNDVI) of the current growing season was obtained, which is recommended for assessing the influence of external factors on the vegetation process. The characteristic ΔNDVI can be used as an independent variable (optimization criterion) in factorial models for predicting the dynamics of the vegetation process.

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