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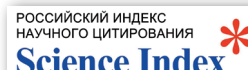
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## МИКРОБИОЦЕНОЗ СОЛОНЦА КАК ИНДИКАТОР ИЗМЕНЕНИЯ СРЕДЫ ПРИ ЗАМЕНЕ КОРМОВЫХ СЕВООБОРОТОВ СЕЯНЫМ ЛУГОМ

Коробова Л.Н.<sup>1</sup>, (✉)Риксен В.С.<sup>2</sup>, Батурина О.А.<sup>3</sup>

<sup>1</sup>Новосибирский государственный аграрный университет  
Новосибирск, Россия

<sup>2</sup>Сибирский федеральный научный центр агробиотехнологий Российской академии наук  
Новосибирская область, р.п. Краснообск, Россия

<sup>3</sup>Институт химической биологии и фундаментальной медицины  
Сибирского отделения Российской академии наук  
Новосибирск, Россия

(✉)e-mail: Riclog@mail.ru

На Барабинской равнине влияние севооборотов с донником и кострцом безостым на солонцы изучается в динамике более 30 лет. Отмечено, что в почве с фитомелиоративными севооборотами общие запасы солей в сравнении с исходной целиной значительно снизились. В слое почвы 0–20 см их количество уменьшилось в 3,8–4,4 раза, в слое 20–40 см – в 4,6–7,7 раза. В результате залужения участков фитомелиоративных севооборотов смесью кострца безостого и люцерны синегибридной эффект рассоления снижается (в верхнем слое в среднем на 6,4 и 9,3%, в нижнем – на 24,9% в последствии севооборота с кострцом). Выявленные изменения в засолении почвы нашли отражение в представительстве солеустойчивых и солечувствительных бактерий. Обилие мало переносящих соль представителей класса спартобактерий на залуженном участке после севооборотов с донником и кострцом снизилось в 3,2 и 3,6 раза, а относительно солелюбивых *Cytophagia* увеличилось в 1,6 и 2,4 раза. В сеяном лугу после севооборота с донником отмечено большее количество сложно разлагаемых растительных остатков (преимущественно злаков), о чем свидетельствует возросшее содержание ацидобактерий. У донника, судя по численности родов *Gaiella* из класса *Thermoleophilia* и *Microfunatus* из класса *Actinobacteria*, мелиоративный эффект в отношении рассоления и аэрации солонца среднего больше, чем у кострца. Залужение увеличивает в солонце среднюю активность минерализации и олиготрофность почвы в слое 20–40 см сильнее, чем в слое 0–20 см в 1,6–2,2 раза. Потенциальное микробиологическое гумусонакопление под сеяным лугом уменьшается в верхнем слое участка, ранее занимаемого севооборотом с донником, и в нижнем слое – севооборотом с кострцом.

**Ключевые слова:** солонец средний, фитомелиорация, кормовые травы, залужение, микробиом, 16S рРНК, биологическая активность почвы

## SOLONETZ MICROBIOCENOSIS AS AN INDICATOR OF ENVIRONMENTAL CHANGE WHEN REPLACING FORAGE CROP ROTATIONS WITH SOWN MEADOW

Korobova L.N.<sup>1</sup>, (✉)Riksen V.S.<sup>2</sup>, Baturina O.A.<sup>3</sup>

<sup>1</sup>Novosibirsk State Agrarian University  
Novosibirsk, Russia

<sup>2</sup>Siberian Federal Research Centre of Agro-BioTechnologies of the Russian Academy of Sciences  
Krasnoobsk, Novosibirsk region, Russia

<sup>3</sup>Institute of Chemical Biology and Fundamental Medicine, Siberian Branch of the Russian Academy of Sciences  
Novosibirsk, Russia

(✉)e-mail: Riclog@mail.ru

In the Baraba Plain, the influence of crop rotations with sweet clover and awnless brome grass on solonets has been studied in dynamics for more than 30 years. It has been noted that in the soil with

phytomeliorative crop rotations total salt reserves have significantly decreased in comparison with the initial virgin soil. In the 0–20 cm soil layer, their number decreased 3.8–4.4 times, in the 20–40 cm layer – 4.6–7.7 times. As a result of grassing of the phytomeliorative crop rotation plots with a mixture of awnless brome grass and alfalfa blue-hybrid, the effect of desalinization is decreasing (in the upper layer on average by 6.4 and 9.3%, in the lower layer – by 24.9% in the aftermath of the crop rotation with awnless brome grass). The identified changes in the soil salinity have been reflected in the representation of salt-tolerant and salt-sensitive bacteria. The abundance of low salt-tolerant representatives of the class Spartobacteria on the grassed area after crop rotations with sweet clover and brome grass decreased by 3.2 and 3.6 times, and the abundance of the relatively salt-loving *Cytophagia* increased by 1.6 and 2.4 times. In the sown meadow after crop rotation with sweet clover, a higher amount of complexly decomposable plant residues (mainly cereals) was observed, as evidenced by the increased content of acidobacteria. According to the abundance of the genera *Gaiella* from the class Thermoleophilia and *Microlunatus* from the class Actinobacteria, the meliorative effect in terms of desalinization and aeration of solonets is greater in sweet clover than in brome grass. Grassing increases mineralization activity and oligotrophic soil in solonetz on average in 20–40 cm layer more strongly than in 0–20 cm layer by 1.6–2.2 times. Potential microbiological humus accumulation under sown meadow decreases in the upper layer of the plot previously occupied by the rotation with sweet clover, and in the lower layer – by the rotation with brome grass.

**Keywords:** medium solonetz, phytomelioration, fodder grasses, grassing, microbiome, 16S rRNA, soil biological activity

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#### Конфликт интересов

Авторы заявляют об отсутствии конфликта интересов.

#### Conflict of interest

The authors declare no conflict of interest.

## INTRODUCTION

Solonetz and alkaline areas of the Baraba steppe are characterized by low productivity of natural lands<sup>1</sup>. To improve it and create a stable fodder base, in the 1980s, scientists developed technologies for growing fodder crops, including a set of drought-, salt-, and solonetz-resistant annual and perennial grasses – phytomeliorants, and agronomic methods of improving the properties of solonetzic soils<sup>2</sup> [1].

One of the first places in Baraba as a phytomeliorant was given to melilot, and among perennial grasses with high fodder merits – awnless brome. In 1987, a station of the Siberian

Research Institute of Fodder Crops (SRI of Fodder Crops) was established on the solonetz in the middle of Baraba, where six-field crop rotations with yellow melilot and awnless brome began. After three rotations under the influence of the agrobiological method, a transformation of the properties of solonetz soils occurred<sup>3</sup> [2], making them suitable for creating a highly productive long-term meadow agroecosystem. Consequently, part of the rotational land was artificially grassed down with a grass mixture of awnless brome and hybrid alfalfa, which was done 13 years prior to these studies. This allowed for a reduction in labor costs while maintaining high

<sup>1</sup>Konstantinov M.D., Kucherenko A.M. Timing and methods of grassing Baraba solonets // Fodder Production, 2000, N 4, pp. 13-15.

<sup>2</sup>Konstantinov M.D., Kukhar M.A. Improvement of the properties of chernozem-meadow fine solonets in phytomeliorative meadow crop rotations in Western Siberia // Proceedings of the Russian Academy of Agricultural Sciences, 2006, N. 6, pp. 31-34.

<sup>3</sup>Konstantinov M.D., Lomova T.G., Kukhar M.A. Phytomeliorative meadow crop rotations on solonetz soils in Western Siberia // Siberian Branch of the Russian Academy of Agricultural Sciences, 2011, 104 p.



yields of the used perennial grasses<sup>4</sup>. At the same time, the task of assessing the duration of the preservation of the positive properties of solonetz soils, acquired under the influence of the agrobiological method of amelioration, became relevant.

Currently, there is no consensus in the literature on this matter. It is possible that the absence of soil tillage over several years and the genetic peculiarities of solonetz soils will gradually lead to the return of the characteristics limiting the productivity of the grass stand. Early stages of this can be detected by studying the soil microbial community – its biological activity and biodiversity. The sensitivity of microflora to the slightest changes in the physical and physico-chemical state of soils has been proven in many scientific studies<sup>5</sup> [3–5]. This has made biological activity and the abundance of individual ecological-trophic groups an important criterion for assessing soil fertility and an indicator of the danger of agrochemicals used in agricultural practice<sup>6</sup>.

By studying the microflora, one can judge the duration of the positive impact on the solonetz of sown perennial grasses (grassing down) in the absence of mechanical destruction of the solonetz horizon and the accompanying improvement of the soil's water-air regime.

The purpose of the study is to compare changes in the rhizosphere microflora of a 13-year-old artificial meadow, devoid of soil tillage, with the state of microflora in the middle solonetz, cultivated with fodder crop rotations.

Research objectives are:

- to study the microbiological component of the middle solonetz;
- to determine the overall salt content in the soil.

## MATERIAL AND METHODS

The study focused on the microflora of gleyic solonetz meadow soil (Gleyic Solonetz Albic.), medium, medium-sodium, columnar with soda-sulfate type of salinization. The research was conducted at the station of the Siberian Research Institute of Fodder Crops of the Siberian Federal Scientific Centre of Agro-BioTechnologies of the Russian Academy of Sciences (SRI of Fodder Crops SFSCA RAS) in the Novosibirsk region (55.389° N, 78.927° E). The experiment included the following variants: 1) yellow melilot in a six-field crop rotation (melilot with a cover crop of Sudan grass – second-year melilot – oats for grain-seed); 2) grassing down with alfalfa and awnless brome after the melilot crop rotation; 3) awnless brome in a six-field crop rotation (millet – millet + brome – 4-year brome); 4) grassing down with alfalfa and awnless brome after the brome crop rotation.

Soil samples were collected in 2016, 2018, 2019, and 2020 after mowing in the first ten-day period of August from the rhizosphere layers of 0–20 and 20–40 cm. For comparison of salt content, soil was also collected in its natural state (layers 0–15 and 15–40 cm). For classical microbiological research, one mixed sample was prepared, and for metagenomic analysis (2020) – four mixed samples. The total salt content in the soil was determined using a KL-C-1 conductometer based on the EC (electrical conductivity) indicator of soil paste diluted with water in a 1:5 ratio<sup>7</sup>. The taxonomic affiliation of bacteria was identified at the Center for Collective Use “Genomics” (Novosibirsk) of the Institute of Cytology and Genetics of the Russian Academy of Sciences using high-throughput sequencing of the V3–V4 region of the 16S rRNA gene. Total DNA was extracted using the DNeasy PowerSoil Kit (Qiagen). For mechani-

<sup>4</sup>Lomova T.G. Transformation of solonchaks into fertile land // Actual problems of agriculture in mountainous areas: proceedings of the VI International Scientific and Practical Conference, Gorno-Altai, June 8-11, 2017, Gorno-Altai: Gorno-Altai State University, 2017, pp. 29-33.

<sup>5</sup>Artamonova V.S. Microbiological features of anthropogenically transformed soils of Western Siberia. Novosibirsk: Publishing House of SB RAS, 2002, 208 p.

<sup>6</sup>Microbiological guidelines for comprehensive monitoring of soil fertility of agricultural soils. Moscow, 2003, 82 p.

<sup>7</sup>Zaidelman F.R., Smirnova L.F., Shvarov A.P., Nikiforova A.S. Practicum on the course “Soil Reclamation”. Moscow: Grif and K. 2008, 66 p.

cal disruption of the sample, TissueLyser II (Qiagen) was used for 10 min at 30 Hz. DNA quality was assessed using 1% agarose gel electrophoresis, and DNA quantity was measured on Quibit (Life Technologies) and Nanodrop (Thermo Fisher Scientific). The V3–V4 region of the 16S rRNA gene was amplified using primers 343F (5'-CTCCTACGGRRSGCAGCAG-3') and 806R (5'-GGACTACNVGGGTWTCTAAT-3'), containing adapter sequences (Illumina), linker, and barcode<sup>8</sup>. The conditions for amplification and analysis of the obtained paired sequences followed those outlined in a previous study [6]. The taxonomic affiliation of OTU sequences was determined using SINTAX [7] with the 16S RDP training set v16 as the reference database<sup>9</sup>.

In classical microbiological research, the number of microorganisms assimilating organic nitrogen (MPA), mineral nitrogen (SAA), and oligonitrophiles (SA) was studied using the method of limiting dilutions on nutrient media. The seeding repeatability for one sample was three and four times. The obtained data were statistically processed, calculating the standard deviation or LSD.

Using the identified abundance of bacteria on MPA and SAA, the coefficient of potential microbiological transformation of organic matter into humus reserves  $P_m = (MPA + SAA) \times (MPA / SAA)^{10}$ , the mineralization coefficient  $K_{min.} = SAA / MPA$ , and the oligotrophy coefficient  $K_{oligotrof} = SA / MPA$  were calculated.

The weather conditions during the years of the study varied significantly. According to the Selyaninov hydrothermal coefficient, 2016 was moist and warm (HTC = 1.01), 2018 was moist and cold (HTC = 1.87), and 2019 and 2020 were dry and warm (HTC = 0.74 and 0.65).

## RESULTS AND DISCUSSION

The phytomeliorative effect of grasses on solonetz soils is largely associated with their ability to extract calcium from the soil, which

replaces sodium in the soil-absorbing complex upon the decay of roots. This results in a change in the salt content in the root zone, determined internationally by the EC (electrical conductivity) indicator.

The changes in the physico-chemical properties of the solonetz transformed by crop rotations and the solonetz after prolonged grassing down according to EC are shown in Fig. 1.

It was found that in soils with phytomeliorative crop rotations (using agronomic measures), the total salt reserves over 30 years significantly decreased compared to the original uncultivated soil. In the 0–20 cm soil layer, their amount decreased by 3.8–4.4 times, and in the 20–40 cm layer, by 4.6–7.7 times. The more marked differences from the uncultivated solonetz in the EC indicator in the lower layer were caused by agrobiological amelioration using awnless brome.

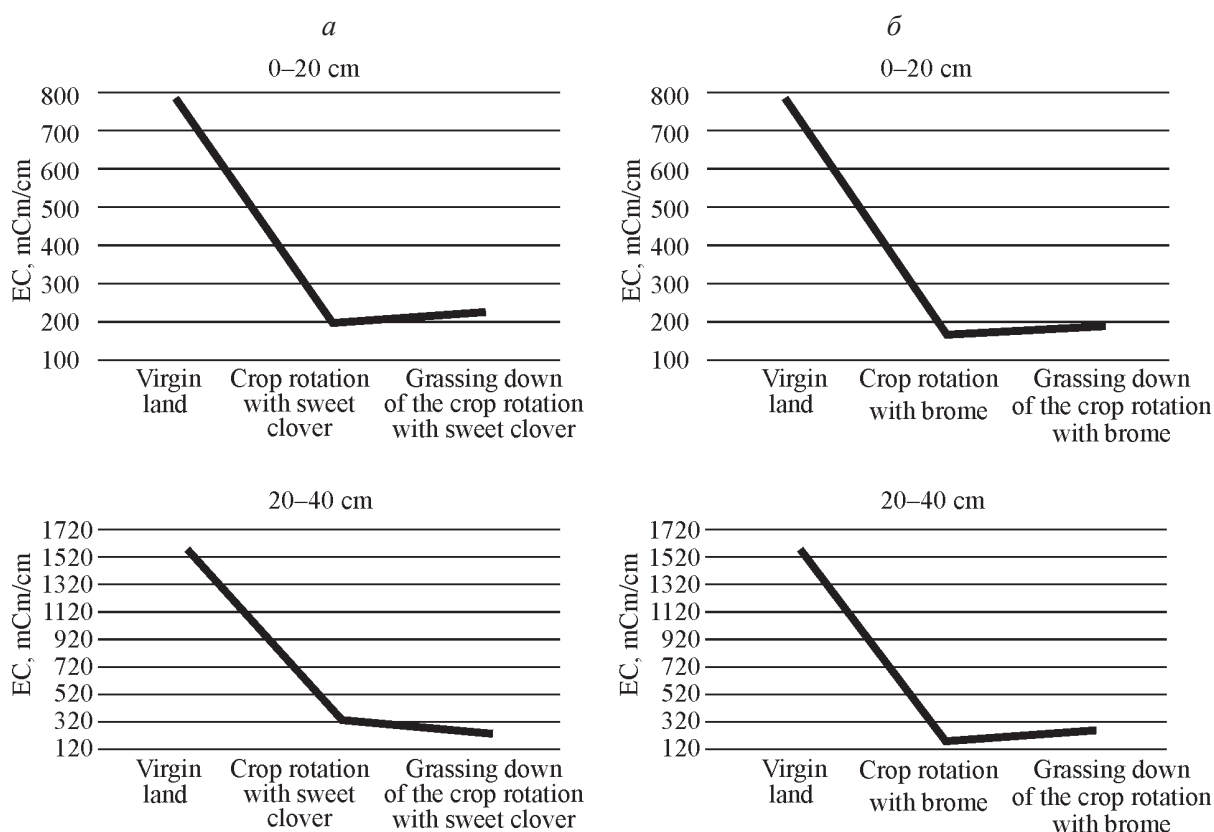
Post-rotation grassing down of part of the territory with crop rotations, conducted in 2007, slightly reduced the desalination effect by the time of these studies. In the 0–20 cm soil layer, this manifested after both crop rotations, but more strongly after the rotation with awnless brome. Here, under the grass mixture of brome and alfalfa, more salts were registered on average by 9.3%. In the solonetz after melilot, the amount of salts on grassing down increased by 6.4%. In the 20–40 cm soil layer, an increase in EC was noted only after the brome crop rotation (by 24.9%). In the variant with grassing down after the melilot rotation, the total amount of salts continued to decrease.

The identified changes in soil salinity under the grass mixture of brome and alfalfa, in the absence of mechanical destruction of the solonetz horizon, were reflected in the representation of salt-resistant and salt-sensitive bacteria. Sequencing of 16S rRNA amplicons revealed six bacterial classes that changed in the rhizosphere of plants under the influence of post-rotation

<sup>8</sup>Fadrosch D.W., Ma B., Gajer P., Sengamalay N., Ott S., Brotman R.M., Ravel J. An improved dual-indexing approach for multiplexed 16S rRNA gene sequencing on the Illumina MiSeq platform // *Microbiome*, 2014, 2(1): 6.

<sup>9</sup>Wang Q., Garrity G.M., Tiedje J.M., Cole J.R. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy // *Applied and environmental microbiology*, 2007, vol. 73, N 16, pp. 5261–5267.

<sup>10</sup>Mukha V.D. About indicators reflecting the intensity and direction of soil processes // *Collection of scientific works of Kharkov Agricultural Institute*, 1980, vol. 273, pp. 13–16.



**Рис. 1.** Изменение содержания солей в солонце луговом гидроморфном среднем под влиянием кормовых севооборотов и их залужения (в среднем за 2018–2020 гг.):

*a* – севооборот с донником; *б* – севооборот с кострцом (в целине почва отобрана из слоев 0–15 и 15–40 см)

**Fig. 1.** Changes in the salt content in the medium meadow hydromorphic solonetz under the influence of fodder crop rotations and their grassing (average for 2018–2020):

*a* – crop rotation with sweet clover; *б* – crop rotation with brome (the soil is sampled from 0–15 and 15–40 cm of virgin soil)

grass seeding (see Table 1). These classes had at least 0.1% of sequences among the four studied variants and four repetitions of each.

The most significant response to grassing down was observed in the classes of acidobacteria from the phylum *Acidobacteria* and spartobacteria from *Verrucomicrobia*. The content of acidobacteria in the solonetz meadow soil, compared to the melilot crop rotation, increased by 1.5 times. They possess a pronounced adaptive ability to obtain nutrients from complex organic substrates<sup>11</sup>. In our case, this is from the residues of cereals after the death of the grass-legume

vegetation of the meadow, which was much less in the rotation of Sudan grass + melilot – second-year melilot – oats. In the solonetz meadow soil after the brome rotation, the number of acidobacteria increased by only by 11%, which also supports the explanation for the increase in acidobacteria on grassing down.

A 3.2–3.6-fold decrease in the number of soil *Spartobacteria* under the sown meadow indicates an increase in the soil alkalinity relative to the solonetz transformed by fodder crop rotations. Spartobacteria have a pronounced intolerance to salt [8], such soil conditions are formed

<sup>11</sup>Ward N.L., Challacombe J.F., Janssen P.H., Henrissat B., Coutinho P.M., Wu M., Kuske C.R. Three genomes from the phylum *Acidobacteria* provide insight into the lifestyles of these microorganisms in soils // *Appl. Environ. Microbiol.*, 2009, vol. 75 (7), pp. 2046–2056.

**Табл. 1.** Относительное обилие классов бактерий, регулируемых постсевооборотным залужением в агрогенно измененном солонце среднем (слой почвы 0–20 см)**Table 1.** Relative abundance of bacterial classes regulated by post-rotation grassing in agrogenerally modified medium solonetz (soil layer 0–20 cm)

Class	Crop rotation with melilot	Aftereffect of the crop rotation with melilot	Crop rotation with brome	Aftereffect of the crop rotation with brome
<i>Acidobacteria</i>	28,1 ± 1,1	42,0 ± 0,8	36,6 ± 1,9	41,3 ± 1,8
<i>Spartobacteria</i>	9,3 ± 1,8	2,6 ± 0,8	13,9 ± 1,3	4,3 ± 0,9
<i>Thermoleophilia</i>	4,2 ± 0,4	6,0 ± 0,3	2,4 ± 0,3	4,9 ± 0,2
<i>Gemmatimonadetes</i>	4,5 ± 0,5	2,1 ± 0,4	3,3 ± 0,2	2,1 ± 0,2
<i>Blastocatellia</i>	3,9 ± 0,1	1,3 ± 0,1	1,9 ± 0,2	1,2 ± 0,2
<i>Cytophagia</i>	0,2 ± 0,04	0,32 ± 0,03	0,12 ± 0,03	0,28 ± 0,07

on grassing down which hinders the reproduction of these bacteria.

Secondary salinization is weaker in solonetz transformed by the melilot rotation and then grassed down than in grassing down after the brome rotation (see Fig. 1). This is also indicated by the representation of *Cytophagia*. It is known that these bacteria are positively and closely associated with the cations of the soil, as well as with the level of salt content in it [9]. In our case, on the sown meadow site, the abundance of rhizosphere *Cytophagia* after the melilot rotation increased only by 1.6 times, and after the brome rotation – by 2.4 times (correlating with the increase in EC values by 6.4 and 9.3%).

With the increasing salt content in the grassed down solonetz, better acid-alkaline conditions are established, and excess moisture is lost. This change can be indicated by the reduced representation of the classes *Blastocatellia* from the phylum *Acidobacteria* [10] and *Gemmatimonadetes* from the same-named phylum<sup>12</sup>. However, the improvement in acid-alkaline balance and aeration, judging by the number of these indicator microorganisms, is more characteristic of the site with post-rotation grassing down with melilot.

The return changes under meadow grass mixture (without mechanical tillage) relative to the soil occupied by forage crop rotations can

be illustrated more clearly by consideration of Actinobacteria phyla. The phyla are among the three dominant phyla in the microbiome and are indicators of soil moisture<sup>13</sup>, aeration and salinity conditions.

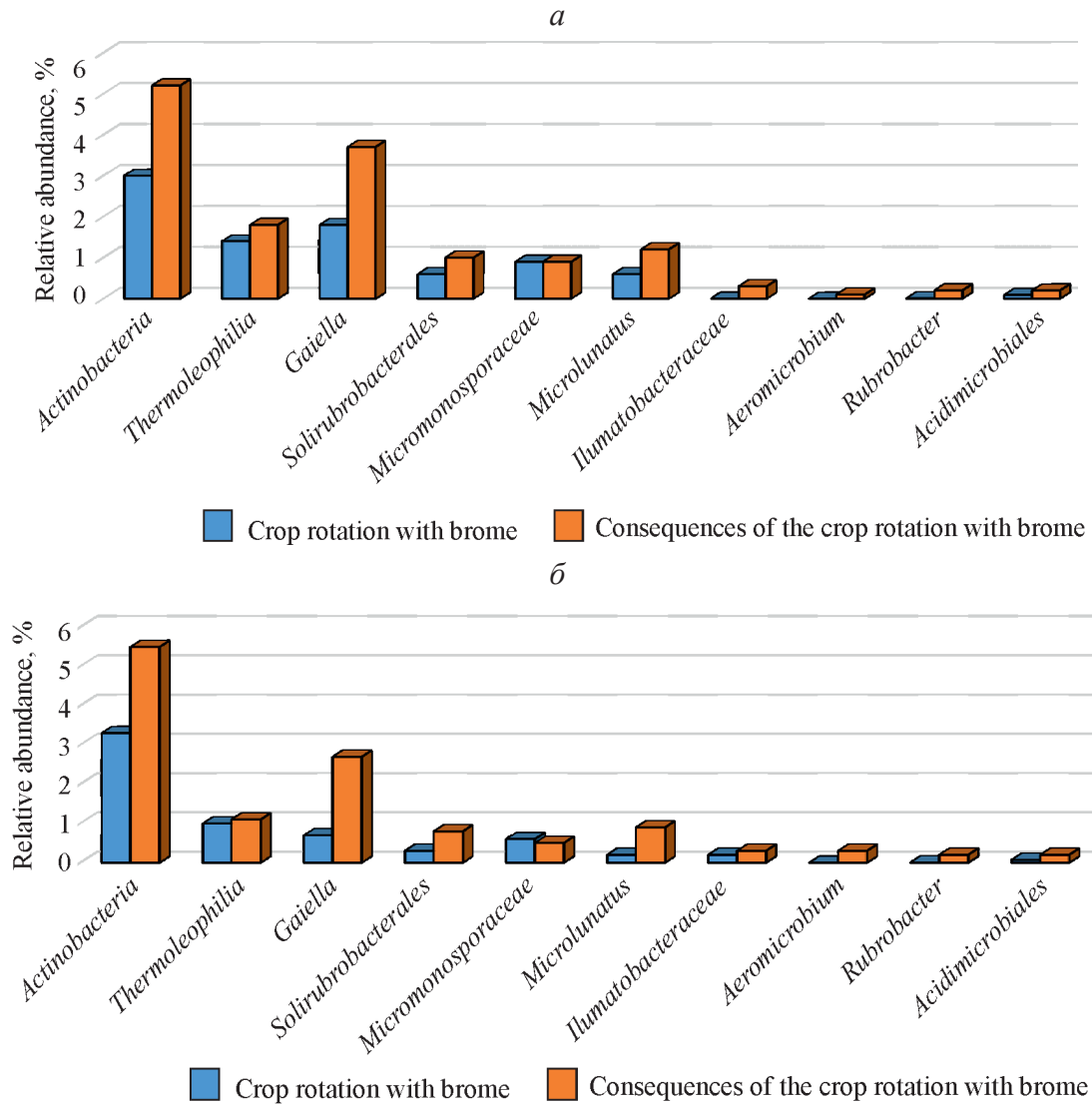
Within this phylum, we identified 10 genera that most noticeably differ in relative abundance between the studied variants (see Fig. 2).

It was found that the genera *Gaiella* from the class *Thermoleophilia* and *Microclunatus* from the class *Actinobacteria* contribute the most to the altered numbers of actinobacteria due to grass seeding. Their abundance on grassing down increases by 2.1 and 4.5 times, and 2.0 and 3.9 times, respectively (see Fig. 2, *a* and *b*). More *Gaiella* bacteria were found in the medium solonetz occupied by melilot, and in its aftermath. Since *Gaiella* are sensitive to the presence of sodium<sup>14</sup>, it can be asserted that melilot has a greater meliorative effect regarding the desalinization of soil than brome. Melilot also loosens the soil more than brome. An indicator of better aeration conditions is the number of actinobacteria from the genus *Microclunatus*. It belongs to the order *Propionibacteriales*, characterized by tolerance to low oxygen concentrations or complete anaerobiosis. The increase in such representatives from this family as *Microclunatus* and *Aeromicrobium* in the agrogenic solonetz on grassing down indicates less oxygen content in

<sup>12</sup>DeBruyn J., Nixon L., Fawaz M., Johnson M., Radosevich M. Global Biogeography and Quantitative Season Dynamics of Gemmatimonadetes in Soil // Appl. Environ. Microbiol., 2011, vol. 77, N 17, pp. 6295–6300.

<sup>13</sup>Chirak E.L., Pershina E.V., Dol'nik A.S., Kutovaya O.V., Vasilenko E.S., Kogut B.M., Merzlyakova Ya.V., Andronov E.E. Taxonomic structure of microbial association in different soils investigated by high-throughput sequencing of 16s-rrna gene library // Agricultural Biology, 2013, N 3, pp. 100–109.

<sup>14</sup>Albuquerque, L., da Costa, M. S. The family gaiellaceae // The prokaryotes, 2014, pp. 357–360.



**Рис. 2.** Разнообразие филои *Actinobacteria* и представительство ее родов в солонце среднем под севооборотами и на залужении (слой 0–20 см):

*a* – влияние севооборота с донником, *б* – влияние севооборота с кострцом

**Fig. 2.** Diversity of the phylum *Actinobacteria* and the representation of its genera in the medium solonetz under crop rotations and on grassing (layer 0–20 cm):

*a* – the effect of the crop rotation with sweet clover, *б* – the effect of the crop rotation with brome

the sown meadow compared to fodder crop rotations, where there is soil treatment, and especially in the aftermath of brome.

Soil microflora is not only an indicative parameter of soil fertility but also participates in its creation. Indicators of optimizing potential soil fertility are considered to be an increase in the reserves of organic matter in the soil (humus formation), and for optimizing effective fertility – the rate of mineralization of organic residues. Both processes are related to microbiological

activity, and the level of their participation is approximately reflected by Pm and Kmin.

The data on mineralization activity indicate that in the medium solonetz under grassing down the decomposition of organic substances in the 0–20 cm soil layer was more active than under crop rotations, by 1.5–2.6 times (see Table 2). This correlates with the change in the abundance of the class Acidobacteria – organic hydrolyzers – in the grassed down variants (see Table 1).

An alternative to reduced mineralization activity is humus accumulation. Due to decreased mineralization activity, the cultivation of grass phytomeliorants in crop rotations contributed to the microbiological transformation of plant residues into soil organic matter. In the “melilot crop rotation” variant, the potential microbiological humus formation (Pm) exceeded the grass seeding variant by 2 times, and in the “brome crop rotation” variant in the 0–20 cm soil layer, it was on the level of the sown meadow.

It should be noted that the upper layer of the medium solonetz under the grass-legume mixture on the sown meadow is more densely populated with oligotrophic microbes: they are more abundant compared to crop rotations by 1.7 and 2.6 times. Oligonitrophiles are present only in environments with residual nitrogen amounts. This means that under crop rotations, copiotrophic soil microflora, inhabiting nutrient-rich soils, predominated. This conclusion is supported by the lesser occurrence of bacteria from the oligotrophic class *Thermoleophilia* in these environments (see Table 1) [11].

The patterns identified in the 0–20 cm layer of the phytomeliorated solonetz in changing microbiological processes also manifested in the 20–40 cm layer. But here, the differences between crop rotations and the sown meadow were stronger. The difference in mineralization coef-

ficients between the grassed down site and the melilot crop rotation reached 3.2 times, and in the oligotrophy coefficient – 3.1 times. Potential humus accumulation (Pm) in the 20–40 cm soil layer corresponded to the 0–20 cm layer.

The difference in mineralization activity between the grassed down site and the brome crop rotation in the lower layer increased to 5.8 times, and in soil oligotrophy – to 4.1 times. Unlike the upper layer of the medium solonetz, in the 20–40 cm layer, differences appeared in the potential microbiological humus accumulation indicator between grassing down and the brome crop rotation – Pm decreased by 2.4 times.

## CONCLUSIONS

1. It has been established that 30 years of cultivating phytomeliorative crop rotations with melilot and brome significantly reduce the total salt reserves in medium solonetz compared to uncultivated land: by 3.8 and 4.4 times in the 0–20 cm soil layer, and by 4.6 and 7.7 times in the 20–40 cm layer, respectively. The more pronounced differences in the EC indicator in the lower layer are caused by the cultivation of awnless brome.

2. Post-rotation grassing down with a brome and alfalfa mixture reduces the desalination effect in solonetz modified by crop rotations. Over 13 years, the EC in the 0–20 cm soil layer in-

**Табл. 2.** Изменение активности микрофлоры в солонце среднем, залуженном после кормовых севооборотов, относительно занятого севооборотами (среднее за 4 года исследований, слои почвы 0–20 и 20–40 см)

**Table 2.** Changes in the activity of microflora in the medium solonetz, grassed after fodder crop rotations, relative to those occupied by crop rotations (average over 4 years of research, layers 0–20 and 20–40 cm)

Indicator	Crop rotation with melilot	Aftereffect of the crop rotation with melilot	Crop rotation with brome	Aftereffect of the crop rotation with brome
<i>Soil layer 0–20 cm</i>				
K mineralization	3,7	9,5*	6,4	9,6*
Pm**	7,1	3,5*	6,3	5,6
K oligotrophicity	0,5	1,3*	1,1	1,9*
<i>Soil layer 20–40 cm</i>				
K mineralization	1,4	4,5*	1,2	6,9*
Pm**	22,3	12,5*	30,7	12,6*
K oligotrophicity	4,3	13,5*	8,7	35,8*

\*  $p_{0.05}$  compared to the variants with crop rotations.

\*\*Ph – coefficient of potential microbiological transformation of organic matter into humus reserves.

creased by 6.4% on the sown meadow site after the melilot rotation and by 9.3% after the brome rotation. In the 20–40 cm soil layer, an increase in EC was noted only following the brome rotation (+24.9%).

3. The classes of bacteria *Spartobacteria* and *Cytophagia* became indicators of the increased soil alkalinity of the original solonetz, transformed by fodder crop rotations, under the sown meadow. The abundance of salt-intolerant spartobacteria in the grassed down site decreased by 3.2 and 3.6 times, while the relative abundance of salt-loving *Cytophagia* increased by 1.6 and 2.4 times after the melilot and brome rotations, respectively.

4. Judging by the abundance of *Gaiella* from the class *Thermoleophilia* and *Microlunatus* from the class *Actinobacteria*, melilot has a greater meliorative effect in terms of desalination and aeration of the soil than brome.

5. In the sown meadow after the melilot rotation, there is an increase in the presence of difficult-to-decompose plant residues (mainly cereals), as indicated by the content of acidobacteria. It increases by 1.5 times compared to the melilot rotation, and by 11% compared to the brome rotation.

6. Grassing down increases the activity of mineralization and oligotrophy in medium solonetz. These changes are characteristic for the 0–20 and 20–40 cm soil layers, but are 1.6–2.2 times more pronounced in the lower soil layer. Grassing down leads to a decrease in potential microbiological humus accumulation. This is evident in the 0–20 cm solonetz layer after the melilot rotation and in the 20–40 cm layer after the brome rotation.

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#### ИНФОРМАЦИЯ ОБ АВТОРАХ

**Коробова Л.Н.**, доктор биологических наук, профессор

✉ **Риксен В.С.**, младший научный сотрудник; **адрес для переписки:** Россия, 630501, Новосибирская область, р.п. Краснообск, а/я 463; e-mail: Riclog@mail.ru

**Батурина О.А.**, младший научный сотрудник

#### AUTHOR INFORMATION

**Larisa N. Korobova**, Doctor of Science in Biology, Professor

✉ **Vera S. Riksen**, Junior Researcher; **address:** PO Box 463, Krasnoobsk, Novosibirsk Region, 630501, Russia; e-mail: Riclog@mail.ru

**Olga A. Baturina**, Junior Researcher

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## АСПЕКТЫ КОМПЛЕКСНОЙ ПЕРЕРАБОТКИ ДАЛЬНЕВОСТОЧНЫХ ЯГОДНЫХ КУЛЬТУР

✉ Разгонова М.П.<sup>1, 2</sup>, Сенотрусова Т.А.<sup>2</sup>, Ли Н.Г.<sup>2</sup>, Тимощенко Е.Е.<sup>3</sup>,  
Мурзина О.Г.<sup>4</sup>, Русакова Е.А.<sup>4</sup>, Голохваст К.С.<sup>1, 5</sup>

<sup>1</sup>Федеральный исследовательский центр «Всероссийский институт генетических ресурсов растений им. Н.И. Вавилова»

Санкт-Петербург, Россия

<sup>2</sup>Дальневосточный федеральный университет, Передовая инженерная школа «Институт биотехнологий, биоинженерии и пищевых систем»

Владивосток, Россия

<sup>3</sup>Магаданский научно-исследовательский институт сельского хозяйства

Магадан, Россия

<sup>4</sup>Камчатский научно-исследовательский институт сельского хозяйства

Камчатский край, Россия

<sup>5</sup>Сибирский федеральный научный центр агробиотехнологий Российской академии наук

Новосибирская область, р.п. Краснообск, Россия

✉ e-mail: Razgonova.mp@dvmfu.ru

Представлено видовое разнообразие дикорастущих ягодных растений Дальнего Востока. Рассмотрены перспективные источники биологически активных веществ, а также щадящие и эффективные способы экстрагирования данных веществ. В Дальневосточном регионе ежегодно произрастает значительное число возобновляемых растительных биоресурсов. Большинство описанных в работе ягодных растений обладают потенциалом для промышленной заготовки. Учеными региона проводятся селекционные работы по сохранению и увеличению сортового разнообразия жимолости камчатской. Продолжается перенос ценных форм жимолости из дикой природы в культуру для включения их в селекционный процесс. Представлены полученные сорта жимолости, их характеристика и урожайность. Растущий интерес вызывают антоциановые пигменты ягод и возможность использования их в качестве натуральных пищевых красителей. К числу видов дикорастущих растений Магаданской области и Чукотского автономного округа, которые представляют собой потенциальные источники антоцианов, относятся голубика болотная (*Vaccinium uliginosum*), различные виды смородины (*Ribes fragrans* P., *R. acidum*, *R. dicuscha*, *R. triste* Pallas), жимолость (*L. chamissoi* Bunge ex kirillon, *L. edulis* Turczaninow ex Greun) и другие дикорастущие ягоды. Значимой задачей является модификация и разработка новых способов экстрагирования биоактивных соединений из растительного сырья. Предложено использование высокоэффективного и экологически безопасного способа экстракции – сверхкритической флюидной CO<sub>2</sub>-экстракции. Использование сверхкритического диоксида углерода в комплексе с другими растворителями позволяет с большей полнотой провести извлечение биологически активных соединений из растительных матриц. Исследования, посвященные интенсификации процессов извлечения экстрактивных соединений из дикорастущих ягод Дальнего Востока и их последующей идентификации, позволят сформировать научно обоснованный комплексный подход к переработке плодово-ягодного дикорастущего сырья для пищевой и биотехнологической промышленности.

**Ключевые слова:** дикорастущие ягодные растения Дальнего Востока, растительные полифенолы, растительные пигменты, антоцианы, антиоксиданты, жимолость

**ASPECTS OF COMPLEX PROCESSING OF FAR EASTERN BERRY CROPS**

✉ **Razgonova M.P.<sup>1,2</sup>, Senotrusova T.A.<sup>2</sup>, Li N.G.<sup>2</sup>, Timoschenko E.E.<sup>3</sup>,  
Murzina O.G.<sup>4</sup>, Rusakova E.A.<sup>4</sup>, Golokhvast K.S.<sup>1,5</sup>**

<sup>1</sup>*Federal Research Center “All-Russian Institute of Plant Genetic Resources named after N.I. Vavilov”  
Saint Petersburg, Russia*

<sup>2</sup>*Far Eastern Federal University, Advanced Engineering School “Institute of Biotechnology,  
Bioengineering and Food Systems”  
Vladivostok, Russia*

<sup>3</sup>*Magadan Research Institute of Agriculture  
Magadan, Russia*

<sup>4</sup>*Kamchatka Research Institute of Agriculture  
Kamchatka Territory, Russia*

<sup>5</sup>*Siberian Federal Scientific Centre of Agro-BioTechnologies of the Russian Academy of Sciences  
Krasnoobsk, Novosibirsk region, Russia*

✉ e-mail: Razgonova.mp@dvfu.ru

The species diversity of wild berry plants of the Far East is presented. Promising sources of biologically active substances, as well as gentle and effective methods of extraction of these substances are considered. A significant number of renewable plant bioresources grow annually in the Far Eastern region, most of the berry plants described in this paper have the potential for industrial harvesting. Scientists of the region carry out breeding works on preservation and increase of the varietal diversity of Kamchatka honeysuckle. The transfer of valuable forms of honeysuckle from the wild to culture for inclusion in the breeding process is in progress. The obtained honeysuckle varieties, their characteristics and yields are presented. There is growing interest in the anthocyanin pigments of the berries and the possibility of using them as natural food colors. Wild plant species of the Magadan region and Chukotka Autonomous Okrug that represent potential sources of anthocyanins include bog blueberry (*Vaccinium uliginosum*), various currant species (*Ribes fragrans* P., *R. acidum*, *R. dicuscha*, *R. triste Pallas*), honeysuckle (*L. chamissoi* Bunge ex Kirillon, *L. edulis* Turczaninow ex Freyn) and other wild berries. A significant task is the modification and development of new methods of extraction of bioactive compounds from plant raw materials. The use of a highly efficient and environmentally safe extraction method – supercritical fluid CO<sub>2</sub> extraction – is proposed. The use of supercritical carbon dioxide in combination with other solvents allows for a more complete extraction of biologically active compounds from plant matrices. Studies devoted to the intensification of the processes of extractive compounds extraction from wild berries of the Far East and their subsequent identification will allow to form a scientifically grounded complex approach to the processing of wild fruit and berry raw materials for food and biotechnological industry.

**Keywords:** wild berry plants of the Far East, plant polyphenols, plant pigments, anthocyanins, antioxidants, honeysuckle

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**Conflict of interest**

The authors declare no conflict of interest.

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One of the popular groups of edible wild plants among the population is berries which are of interest as prospective raw materials for the food, biotechnological, cosmeceutical, pharmaceutical, and other industries. Among these plants, the following species are known for their pleasant organoleptic characteristics: wild strawberry, Juneberry, bilberry, blackberry, raspberry, etc.<sup>1</sup> [1, 2].

Most wild berries and fruits are rich sources of bioactive substances (vitamins, polyphenolic complexes, macro- and microelements, etc.). For example, cranberry and wild bilberry have a high content of flavonoids, which can have a protective effect on the human body and be used as nutraceuticals and functional food ingredients [1, 3]. In the Far Eastern region of Russia, a significant number of renewable plant bioresources grow annually. The biological reserves of berry fruits in the Magadan region and the Kamchatka territory, as well as the extent of their use by humans, are crucial characteristics for assessing the resource potential and the possibility of comprehensive processing, including use in agro- and food biotechnologies.

Industrial processing of berry fruits involves a comprehensive approach, which is important for the rational use of biological resources. It is promising to obtain bioactive substances (BAS) from berry fruits, including those not suitable for consumption based on organoleptic characteristics. In this context, it is necessary to determine the most promising method of extracting BAS, including polyphenolic complexes, that will preserve the properties of BAS and ensure an adequate yield of substances. In identifying the most promising method of extraction, there are several disadvantages and limitations: for example, BAS from plant matrices are quite dif-

ficult to extract due to their low mass fraction; examples of such plant matrices include essential oil and medicinal plants. It is also important to consider limitations and drawbacks in sample preparation during liquid extraction: the lengthy process, low productivity, the need for additional freeing of target substances from solvents not permitted for use in the food and feed industries, low selectivity and limited control options during extraction, using only a small part of the extract for analysis, etc.

In this regard, the use of supercritical fluid extraction is promising. Compared to other methods, this approach has several advantages: the ability to conduct selective extraction by changing the temperature and pressure of the fluid; reducing the risk of distorting the sample composition due to various impurities brought in with the solvent (carbon dioxide, nitrogen oxide (I)) because its purity is significantly higher than any organic solvent; simplifying the process of extracting and retrieving target substances from the extract<sup>2,3</sup>.

To utilize bioactive substances (BAS) obtained from extracts in agro- and food biotechnologies, it is important to use the safest possible extractants for humans and animals. Compared to other potential supercritical solvents, carbon dioxide is non-toxic, non-flammable, environmentally friendly, and a renewable resource (see footnotes 2, 3). As a result, supercritical fluid CO<sub>2</sub> extraction is a promising method.

The purpose of the study is to examine the biopotential of berry crops in the Magadan region and the Kamchatka territory.

Standard research methods have been applied, including analytical selection, propagation by herbaceous cuttings “with the bottom” and hardwood cuttings with a growing point.

<sup>1</sup>*Khokhryakov A.P.* Analysis of the flora of the Kolyma Plateau / ed. by V.N. Pavlov; Reports of the Academy of Sciences, Institute of Biological Problems of the North. Moscow: Nauka, 1989, 152 p.

<sup>2</sup>*Kasyanov G.I., Stasieva O.N., Latin N.N.* Pre- and supercritical extraction: advantages and disadvantages // Food Industry. 2005, N 1, pp. 36-39.

<sup>3</sup>*Pokrovsky O.* Sample preparation in chemical analysis by supercritical fluid extraction method // Methodology. 2013, N 6, pp. 22–27.

The content of dry substances (%) was determined by thermogravimetric method according to GOST 29031-91, soluble sugars (%) by the permanganate method according to GOST 8756.13-87, and ascorbic acid (%) by the titrimetric method converted to citric acid according to GOST 24556-89.

Currently, wild berry plants from the Magadan region, which are promising for use

in food biotechnologies, have been described. Their appearance is presented in Figures 1–8.

Berry plants growing on the peninsula are also of interest (see Figures 9–13).

The annual biological reserve of the most common wild edible berry plants in the Magadan region is estimated to be around 130 thousand tons – accounting for 6.2% of the total berry reserve in the entire Far Eastern region of Russia; in the Chukotka region, the berry reserves of the



**Рис. 1.** Брусника (*Rhodococcum avrorin*)

**Fig. 1.** Lingonberry (*Rhodococcum avrorin*)



**Рис. 4.** Рябина сибирская (*Sorbus sibirica* Hedlung)

**Fig. 4.** Siberian mountain ash (*Sorbus sibirica* Hedlung)



**Рис. 2.** Голубика (*Vaccinium uliginosum*)

**Fig. 2.** Bog Blueberry (*Vaccinium uliginosum*)



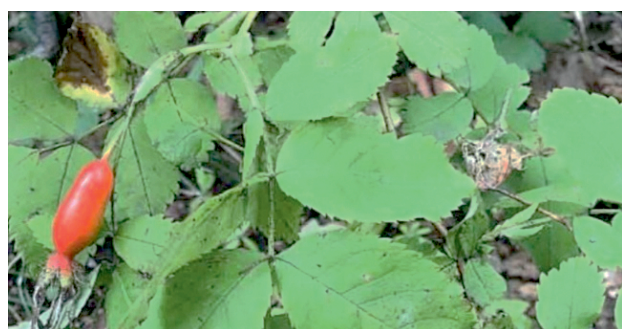
**Рис. 5.** Черемуха (*Padus* Miller)

**Fig. 5.** Bird cherry (*Padus* Miller)



**Рис. 3.** Малина обыкновенная (*Rubus stellatus* Smith)

**Fig. 3.** Common raspberry (*Rubus stellatus* Smith)



**Рис. 6.** Шиповник (*Rosa jacutica* Juzepczuk)

**Fig. 6.** Wild rose (*Rosa jacutica* Juzepczuk)



**Рис. 7.** Смородина черная (*Ribes nigrum* Sibilla)  
**Fig. 7.** Black currant (*Ribes nigrum* Sibilla)



**Рис. 8.** Смородина печальная (*Ribes triste* Pallas)  
**Fig. 8.** Swamp red currant (*Ribes triste* Pallas)

production fund are about 14.5 thousand tons – 1% of the total berry reserve in the entire Far Eastern region [2, 4].

It is known that the fruits and berries of wild berry plants, such as currants, are rich in provitamins, vitamins, polyphenolic complexes, etc. (see Table 1).

It can be concluded that the raw material potential of wild berries in the Far Eastern region has sufficient volumes for industrial processing



**Рис. 9.** Малина приземистая, морошка (*Rubus chamaemorus* L.)  
**Fig. 9.** Fen berry, cloudberry (*Rubus chamaemorus* L.)



**Рис. 10.** Шикша водяная, сибирская (*Empetrum sibiricum* V. Vasiliev)

**Fig. 10.** Water crowberry, Siberian (*Empetrum sibiricum* V. Vasiliev)



**Рис. 11.** Смородина дикуша, алданский виноград (*Ribes dicuscha* Fischer ex Turczaninow)

**Fig. 11.** Dikusha currant, Aldan grape (*Ribes dicuscha* Fischer ex Turczaninow)



**Рис. 12.** Жимолость съедобная, камчатская (*Lonicera edulis* Turczaninow ex Freyn)

**Fig. 12.** Edible honeysuckle, Kamchatka (*Lonicera edulis* Turczaninow ex Freyn)



**Рис. 13.** Шиповник (*Rosa acicularis* Lindley)  
**Fig. 13.** Wild rose (*Rosa acicularis* Lindley)

to extract bioactive compounds and their use in the development of dietary supplements, as well as in food biotechnology.

A significant place in the cultivation and selection of berries in the Far Eastern region is occupied by the experimental site of the Kamchatka Research Institute of Agriculture, located in the south-eastern zone of the Kamchatka Peninsula. Here, long-term research is conducted on Kamchatka honeysuckle (*Lonicera kamtschatica* (Sevast.) Pojark), which is found in all districts of the territory<sup>4</sup> [5].

Results of modern scientific research show the high nutritional value and preventive properties of Kamchatka honeysuckle. Its fruits contain vitamin C, carotenoids, B vitamins, and P-active polyphenols, which determine its high antioxidant activity. The dessert flavor of the fruits of certain species and forms of honeysuckle is due to the increased sugar content with relatively low acidity. The sugar composition includes glucose, fructose, galactose, and rhamnose. The fruits of the honeysuckle contain macroelements such as potassium, phosphorus, calcium, sodium, magnesium, iron, silicon, as well as trace elements copper, zinc, strontium, barium, and iodine [3, 5–7].

At the Kamchatka Research Institute of Agriculture, research work on studying the initial material of honeysuckle and identifying high-yielding forms for introducing honeysuck-

le into cultivation is carried out using the method of analytical selection (see Fig. 14).

Currently, the transfer of valuable honeysuckle forms from the wild into cultivation continues for their inclusion in the breeding process. The main goal of breeding Kamchatka honeysuckle is to preserve and mobilize its biodiversity. This task is particularly relevant due to the increasing anthropogenic pressure on the natural habitats of honeysuckle in Kamchatka [8, 9]. The goal of the breeding work at the Kamchatka Research Institute of Agriculture is to improve the assortment of honeysuckle and create new promising early-fruiting varieties with high productivity and fruit quality.

New varieties should be characterized by frost resistance, various ripening times, high yield (1.5–2.5 kg per bush), early fruiting onset (in the 3rd year after planting), attractive, large (fruit weight over 1 g), non-shedding fruits with easy detachment, a dessert flavor, high content

**Табл. 1.** Химический состав смородины (*Ribes vulgare*), %

**Table 1.** Currant chemical composition (*Ribes vulgare*), %

Indicator	Content
Solids	14,26 ± 0,26
Carbohydrates	6,33 ± 0,21
Ascorbic acid	34,83 ± 5,24
Soluble pectins	0,13–0,18 ± 0,04



**Рис. 14.** Селекционный питомник Камчатского НИИСХ

**Fig. 14.** Selection nursery of the Kamchatka Research Institute of Agriculture

<sup>4</sup> *Nechaev A.A.* Species composition, resources and development of wild berry plants of the Russian Far East // *Forestry Bulletin*, 2012, N 3, pp. 127–131.

of sugars, vitamin C, P-active substances, and low acidity. The research work is conducted according to the “Program and Methods of Breeding of Fruit, Berry, and Nut Crops,” “Program and Methods of Variety Testing of Fruit, Berry, and Nut Crops,” “Methods of Biochemical Research of Plants,” and the “Classifier of the genus *Lonicera* L. subsection *Caeruleae* Rehd” [7–10]. The main biochemical compounds in honeysuckle berries are evaluated based on the following parameters: content of dry substances (%) by thermogravimetric method, soluble sugars (%) by permanganate method, and ascorbic acid (%) by titrimetric method converted to citric acid.

As a result of many years of work, 4000 seedlings from various locations in the Kamchatka Territory have been studied, and a collection of 37 introduced varieties obtained from other breeding institutions in Russia has been researched (Federal Research Center “N.I. Vavilov All-Russian Institute of Plant Genetic Resources”, Federal Altai Scientific Centre of Agro-BioTechnologies, regional state unitary enterprise “Bakcharskoe”). From the assessment of the collection study, the best varieties of non-local breeding of honeysuckle have been identified. Particular attention is paid to the development of cultivation techniques for honeysuckle and identifying the most effective methods of propagation in the conditions of the Kamchatka Territory. As a result of studying methods of cutting, effective methods of propagation by herbaceous cuttings “with the bottom” and hardwood cuttings with a growing point have been identified.

Currently, the breeding material is systematically updated, new nurseries have been established, where more than 500 seedlings of wild forms of plantings from 2018-2022 are being studied. For primary variety testing based on a set of economically valuable characteristics, 37 promising selectionally significant forms have been selected into the elite group (see Table 2).

Selectionally significant forms of honey-

suckle are rich sources of bioactive substances, including essential nutrients. Consuming 100 grams of honeysuckle meets 62% of the adequate daily intake level of ascorbic acid.

#### *Kamchatka Honeysuckle Varieties*

*Atlant* – a seedling of Kamchatka honeysuckle from open pollination. An early ripening variety. The yield in the 6th year after planting is 1.0–1.2 kg per bush. The fruits are large (1.2 g), oval-shaped, with a slightly bumpy surface, thin skin, and tender pulp consistency. Easy, dry detachment. The taste is sour-sweet with a pleasant aroma. Dry matter content – 13.16%, sugars – 6.78–9.05, ascorbic acid – 48.90–63.36% (see Fig. 15).

*Slastena* – a seedling of Kamchatka honeysuckle from open pollination. An early ripening variety. The yield in the 6th year after planting is 1.0 kg per bush. The fruits are large (1.6 g), elongated-oval in shape. High taste quality. Sugar content – 7.91%, dry substances – 12.91, ascorbic acid – 36.96–52.80%.

*Sopernitsa Goryanka* – a seedling of Altai honeysuckle from open pollination. A late-ripening variety. The yield in the 6th year after planting is 0.8–1.0 kg per bush. The fruits are medium-sized (0.66–0.92 g), elongated-oval. The taste is sweetish with a noticeable bit-

**Табл. 2.** Характеристика перспективных селекционно значимых форм жимолости

**Table 2.** Characteristics of the promising breeding significant forms of honeysuckle

Indicator	Characteristics
Properties of the promising breeding significant forms of honeysuckle (Kamchatka NIISKh)	High winter hardiness, early and mid-early ripening period, pleasant in taste characteristics, attractive nonshattering fruits
Fruit weight	1,0–1,2 ± 0,03 г
Ascorbic acid	50,8–56,9 ± 1,07%
Solids	15,2–15,6 ± 0,92%
Soluble sugars	7,8–10,5 ± 0,41%
Organic acids	1,8–2,5 ± 0,06%

terness. Fruit detachment is slightly difficult. Dry substance content – 13.98%, sugars – 6.06–8.53, ascorbic acid – 21.10–38.72%.

*Mil'kovchanka* – a seedling of Kamchatka honeysuckle from open pollination. A mid-early ripening variety. The yield in the 6th year after planting is 0.8–1.2 kg per bush. The fruits are large (1.2 g), oval in shape. Dessert taste. Easy, dry detachment. Dry substance content – 12.5%, sugars – 7.0–9.5, ascorbic acid – 38.7–50.9, acidity – 1.7–2.4%.

*Darinka* – a seedling of Kamchatka honeysuckle from open pollination. An early ripening variety. The yield in the 6th year after planting is 1.2 kg per bush. The fruits are large (1.0–1.2 g), elongated-oval. Sour-sweet taste with a refreshing aroma. Easy, dry detachment. Dry substance content – 12.9%, sugars – 9.6, ascorbic acid – 50.8, acidity – 1.7%.

*Elena* – a seedling of Kamchatka honeysuckle from open pollination. An early ripening variety. The yield in the 6th year after planting is 1.7 kg per bush. The fruits are large (1.1–1.3 g), elongated-oval, teardrop-shaped. Slightly diffi-

cult detachment without skin tearing. Dessert taste. Sugar content – 9.4%, ascorbic acid – 47.8, acidity – 1.5%.

In 2022, as a result of many years of work, three new promising elite forms were identified and are being prepared for state variety testing.

*Elite form 1-5 (Malka variety)* – a seedling of Kamchatka honeysuckle from open pollination. An early ripening variety. Productivity is higher than the standard by 0.12 kg/bush (an increase of 31.6%). The fruits are large (1.3 g), wide-pyxidate -shaped. Dessert taste with a distinct aroma. Easy, dry detachment. Sugar content – 8.9%, ascorbic acid – 50.85, dry matter – 14.7%.

*Elite form 1-20 (Vilyuyka variety)* – a seedling of Kamchatka honeysuckle from open pollination. A mid-early ripening variety. Productivity is higher than the standard by 0.14 kg/bush (an increase of 38.8%). The fruits are large (1.1 g), wide-spindle-shaped. Sour-sweet taste with a light piquant bitterness and distinct aroma. Slightly difficult detachment without skin tearing. Sugar content – 7.2%, ascorbic acid – 46.09, dry matter – 12.4%.

*Elite form 31-35 (Ganalochka variety)* – a seedling of Kamchatka honeysuckle from open pollination. An early ripening variety. Productivity is higher than the standard by 0.07 kg/bush (an increase of 18.4%). The fruits are large (1.2 g), elongated-oval in shape, attractive. Sour-sweet, dessert taste with a pronounced aroma. Easy, dry detachment. Sugar content – 8.5%, ascorbic acid – 47.46, dry matter – 15.3%.

It is known that polyphenolic compounds are present in many plants and berries. For example, anthocyanins give bright colors to fruits, berries, and vegetables, and also contribute to taste and aroma. A meta-analysis of eight scientific studies showed that increasing the intake of foods high in polyphenols by 300 mg per day reduces the risk of developing type 2 diabetes [5–7]. Other authors' research suggests that due to their antioxidant properties, polyphenols may be used in treating certain cancers of the gastrointestinal tract [5–7]. Research by Hossain et



**Рис. 15.** Жимолость сорта Атлант  
**Fig. 15.** Atlant honeysuckle



al., 2016, shows that consumption of polyphenol-containing products helps in the prevention and fight against obesity. Scientists suggest that polyphenols suppress the activity of fat tissue cells responsible for inflammation [5–7].

Currently, there is an increasing demand among producers and consumers for natural food colorants, among which red dyes hold leading positions in terms of sales volume. One of the most common among them are anthocyanin pigments. Among the species of plants in the Magadan region and the Chukotka Autonomous District that are potential sources of anthocyanins are bog whortleberry (*Vaccinium uliginosum*), various types of currant (*Ribes fragrans* P., *R. acidum*, *R. ducuscha*, *R. triste* Pallas), honeysuckle (*L. chamissoi* Bunge ex Kirillon, *L. edulis* Turczaninow ex Freyn), and other wild berries.

There is particular interest among researchers in improving and developing new methods of extracting anthocyanins from plant material. Choosing a suitable extraction method considering optimal combinations of extraction factors for obtaining natural anthocyanin dyes is crucial for the success of their isolation. Several methods of extracting anthocyanins from plant material are described in literature sources: maceration, thermal extraction, catalyst-solvent extraction, ultrasonic extraction, microwave-assisted extraction, and supercritical fluid extraction. A promising direction in the field of extracting the polyphenolic complex is supercritical fluid extraction, which has been used since the late 1970s for food analysis, extraction of bioactive substances, determining lipid levels in food products, and levels of toxic substances. Supercritical fluid CO<sub>2</sub> extraction has several advantages: easy removal of the solvent from the final product, high selectivity, and the use of moderate temperatures during extraction, which are important factors when determining the

method of extraction and conducting research in the food and pharmaceutical industries<sup>5</sup> [11, 12].

An alternative to using co-solvents in the case of poorly soluble or practically insoluble compounds is a complete change in the process scheme using so-called solvent-aided supercritical extraction (SAE). Industrial devices with technological schemes containing CO<sub>2</sub> processing installations have already been developed, thus regenerating a large part of the solvent/anti-solvent. The improvement of solvent-aided supercritical extraction is associated with the same process conditions: pressure, temperature, and concentration of dissolved substances in the suspension. However, the main parameter is the molar fraction of CO<sub>2</sub>. This parameter depends on the relative flow rate of CO<sub>2</sub> and liquid solvent to establish a supercritical composition of the precipitate for the used CO<sub>2</sub>/solvent mixture [12]. Comparing possible supercritical solvents, carbon dioxide has the most attractive advantages: non-toxicity, non-flammability, environmental friendliness, and renewability of the resource (see footnote 5) [11–13].

## CONCLUSIONS

Currently, there is increasing public interest in ensuring a complete nutritional diet, as well as in maintaining the necessary quality of products under a sanction regime. Studying the characteristics of the composition of plant berry resources is necessary for their comprehensive processing and the development of new food ingredients based on them. In this context, one of the relevant research directions in the field of food biotechnologies is fundamental and applied scientific research on the chemical composition and properties of wild berry plants, as well as the improvement of processes for obtaining bioactive substances. The use of supercritical fluid CO<sub>2</sub> extraction is promising. Wild

<sup>5</sup>Program and methodology of varietal study of fruit, berry and nut crops / edited by E.N. Sedov and T.P. Ogoltsova. Sedov and T.P. Ogoltsova. Orel: VNIISPК, 1999, 608 p.

berry plants are an important source of bioactive substances, including vitamins, polyphenolic, and mineral complexes. This raw material can be used to obtain food and technological additives for import substitution, which is currently of particular relevance.

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## ИНФОРМАЦИЯ ОБ АВТОРАХ

✉ **Разгонова М.П.**, кандидат, технических наук, доцент, директор; **адрес для переписки:** Россия, 190031, Санкт-Петербург, ул. Большая Морская, 42; e-mail: m.razgonova@vir.nw.ru

**Сенотрусова Т.А.**, кандидат технических наук, доцент

**Ли Н.Г.**, кандидат технических наук, доцент

**Тимошенко Е.Е.**, лаборант

**Мурзина О.Г.**, младший научный сотрудник

**Русакова Е.А.**, младший научный сотрудник

**Голохваст К.С.**, доктор биологических наук, член-корреспондент РАО, профессор РАН, директор

## AUTHOR INFORMATION

✉ **Maya P. Razgonova**, Candidate of Science in Engineering, Associate Professor, Director; **Address:** 42, Bolshaya Morskaya St., Saint Petersburg, 190031, Russia; e-mail: m.razgonova@vir.nw.ru

**Tamara A. Senotrusova**, Candidate of Science in Engineering, Associate Professor

**Natalia G. Li**, Candidate of Science in Engineering, Associate Professor

**Elena E. Timoshchenko**, Laboratory Assistant

**Olga G. Murzina**, Junior Researcher

**Elena A. Rusakova**, Junior Researcher

**Kirill S. Golokhvast**, Doctor of Science in Biology, Corresponding Member RAE, Professor RAS, Director

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## ЭКОЛОГО-БИОЛОГИЧЕСКИЕ ОСОБЕННОСТИ И МЕДОНОСНАЯ ЦЕННОСТЬ ВЯЗОВНИКОВ

✉ Самсонова И.Д.<sup>1,2</sup>

<sup>1</sup> Санкт-Петербургский государственный лесотехнический университет им. С.М. Кирова  
Санкт-Петербург, Россия

<sup>2</sup> Башкирский государственный педагогический университет им. М. Акмуллы  
Уфа, Россия

✉ e-mail: isamsonova18@mail.ru

Медоносные угодья и природно-климатические условия в лесном фонде степного Придонья характеризуются большой изменчивостью. Насаждения вяза гладкого, ильма и береста занимают 2,2% площади степного Придонья. Цель данного исследования – выявить медоносные виды по компонентам лесного фитоценоза в вязовниках байрачных и пойменных и определить медовую продуктивность растительных формаций. Использованы принятые методы научных исследований в пчеловодстве. Получены лесоводственно-таксационные характеристики древостоя, уточнен видовой состав медоносной флоры и определена медовая продуктивность по типам леса. Медовая продуктивность растительной формации байрачных вязовников составляет 106,4–203,3 кг/га. На теневых берегах байрачных дубняков медовую продуктивностью 32,9 кг/га в лесном фитоценозе обеспечивает вяз обыкновенный (*Ulmus laevis*). На присетевом склоне данному значительному показателю (203,3 кг/га) в составе древостоя способствует клен полевой (*Acer campestre*). На световых берегах существенной медовой продуктивностью отличаются медоносы подлеска, которые встречаются преимущественно на лесных полянах и опушках леса, а также представители травянистого покрова. В вязовнике разнотравно-крапиво-ежевиковом пойменных лесов основной медосбор (35,3 кг/га) дают вяз обыкновенный и ива белая (*Salix alba*), в подлеске – крушина слабительная (*Rhamnus cathartica*), ольха (*R. alnus*), калина обыкновенная (*Viburnum opulus*). При умелом использовании медоносного потенциала лесных растений не только возможно успешно развивать пчеловодство, но и обеспечивать значительное увеличение лесного фонда южных регионов Европейской части Российской Федерации.

**Ключевые слова:** вяз гладкий, берест, байрачные вязовники, пойменные леса, медоносные растения, медовая продуктивность, растительные формации, компоненты лесного фитоценоза

## ECOLOGICAL AND BIOLOGICAL FEATURES AND NECTARIFEROUS VALUE OF ELM TREES

✉ Samsonova I.D.

Saint-Petersburg State Forest Technical University  
Saint-Petersburg, Russia

Bashkir State Pedagogical University named after M. Akmulla  
Ufa, Russia

✉ e-mail: isamsonova18@mail.ru

Bee pasturages and natural-climatic conditions in the forest fund of the steppe Pridonye are characterized by great variability. Plantations of European white elm, Scotch elm and English elm occupy 2.2% of the area of the steppe Pridonye. The purpose of this study was to identify honey-bearing species by the forest phytocenosis components in ravine elms and floodplain forests and to determine the honey productivity of plant formations. Accepted methods of scientific research in beekeeping were utilized. The silvicultural and taxational characteristics of the forest stand were obtained, the species composition of the honey-bearing flora was specified and the honey productivity by forest types was determined. Honey productivity of the plant formation of ravine elms is 106.4–203.3 kg/ha. European white elm (*Ulmus laevis*) provides honey productivity of 32.9 kg/ha in the forest phytocenosis on the shady banks of the ravine oak forests. Field maple (*Acer campestre*) contributes to this significant index (203.3 kg/ha) in the stand composition on high and steep gully slopes. On the light banks honey

production is characterized by honey-bearing undergrowth species, which are found mainly in forest glades and woodsides, as well as the carpet plants representatives. In elm-herbage-nettle-blackberry floodplain forests, the main honey yield (35.3 kg/ha) is given by European white elm and white willow (*Salix alba*), while in the undergrowth it is given by common buckthorn (*Rhamnus cathartica*), alder (*R. alnus*), and European cranberry bush (*Viburnum opulus*). Skillful use of the honey-bearing potential of forest plants not only makes it possible to successfully develop beekeeping, but also to ensure a significant increase in the forest fund of the southern regions of the European part of the Russian Federation.

**Keywords:** European white elm, English elm, ravine elms, floodplain forests, nectariferous plants, honey productivity, plant formations, components of forest phytocenosis

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**Конфликт интересов**

Автор заявляет об отсутствии конфликта интересов.

**Conflict of interest**

The author declares no conflict of interest.

## INTRODUCTION

Foreign and domestic scientists in scientific works explore the issues of studying the mutually beneficial influence of the symbiosis between forests and entomophagous insects [1–8]. The use of the resource potential of forest lands for honey collection systematically requires clarification and assessment of honey-producing plant communities. A territory is considered valuable for beekeeping where a variety of natural vegetation is present within the effective flight radius of bees, providing them with nectar and pollen throughout the honey collection season. The more diverse and frequently intersected the area is with beams and valleys, the richer it is in terms of honey production and, under favorable climatic conditions, ensures the best productive honey collection. In the floodplains of rivers and along the ravines of the south of the European part of Russia, such territories occupy a small area and are not of significant importance, although here are found good honey plants: wild fruit and berry species, willows, maples, ringed sage, melilot, clovers, bluegrass, etc.<sup>1</sup> [9].

Studying the honey potential of a specific region is necessary for organizing intensive technologies

for keeping bee families, allowing the rational use of local honey resources [10].

Successional processes in floodplain forests are influenced by the water regime, the intensity of alluvial processes, and geomorphological structure. Elm stands are transitional to oak groves and aspen forests of the central floodplain. For example, on fertile alluvial loams, the intensity of species replacement is maximal. This is most pronounced in oak groves, where there has been a 25% replacement of oak by elm. Along with ecological and anthropogenic factors, the biological properties of tree species significantly affect the transformation of floodplain forest biogeocenoses.

The specificity of the forest-growing conditions of river floodplains has led to a strict correlation of tree species with soils of different mechanical compositions, certain relief elements, flooding zones, and parts of floodplains. Among the formations of floodplain forests, a significant part (54 species) is accounted for by honey plants. On the tops of ridges and along the riverside bank, pollen plants of black poplars grow; low-lying locations and oxbows are occupied by nectar and pollen plants of white willow, coastal and sandy spits are forested with

<sup>1</sup>Gryazkin A.V., Smirnov A.A., Mannapov A.G., Beljaev N.V. Bioresource potential of forest lands as the source of honey yield in steppe area of the river Don // Forests of Russia: policy, industry, science and education. IOP Conference Series: Earth and Environmental Science. IV scientific-technical conference 2019. P. 012057. DOI: 10.1088/1755-1315/316/1/012057.

multi-species formations of willow plots; formations of elm stands and oak groves have gained the most distribution near the central part of the floodplain. In the central part of the floodplain, on the most fertile and rich soils, oak groves and their companions are concentrated. In the terrace-edge part, black alder, a pollen carrier, dominates, and on micro-elevations, there are stands of willow and white poplar.

Elm, elm stands, and elm forests in the area represent 15.6% in relation to the total forest-covered area. The role of elm forests is increasing in the lower reaches of the Don River – accounting for 25.7% [11].

In the Don floodplains, honey-producing lands distinguished by high productivity have been noted. These include shrubby thickets of black maple and willows, as well as the early spring honey plant sloe, which is valuable for honey collection when it spreads in dense thickets of blackthorn.

Plantations of European white elm, Scotch elm and English elm occupy over 10,000 hectares (2.2%) of the area in the steppe region near the Don, predominantly preserved in more humid areas (I–III) (see Fig. 1). Their increase in area in region VIII is associated with artificial introduction into floodplain

cultures. English elm occupies a significant area (20%) in region VII. For elm forests, the predominance of young growth is characteristic, explained by the widespread drying of mature and ripening stands due to Dutch elm disease and the replacement of species in oak clearings due to abundant seedling and seed regeneration of elm.

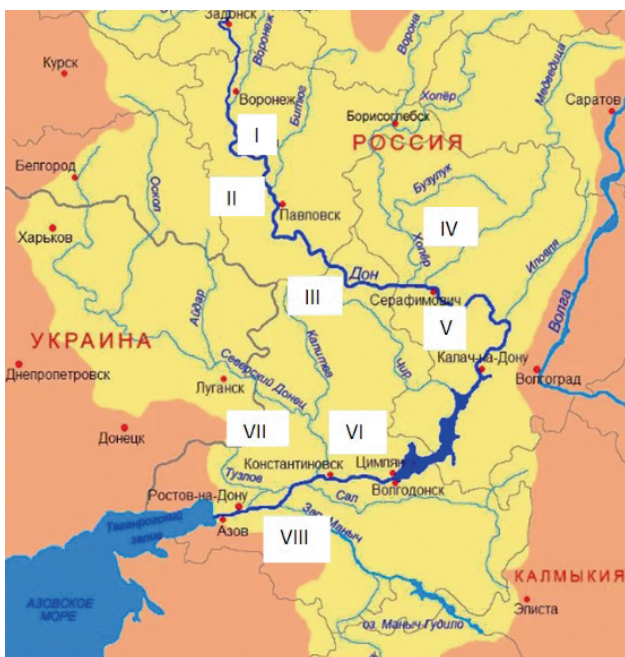
Scientists are currently actively studying the growth characteristics of dwarf elm. V.V. Lepesko and L.P. Rybashlykova note that the condition of tree stands is determined by two complexes of factors: zonal-edaphic and pastoral-economic. The longevity of dwarf elm in the arid zone on the brown soils of the Astrakhan semi-desert is determined by the presence of productive moisture in the soil and its salinity level. It has been established that the vitality of 57–64-year-old dwarf elm stands is noted on sandy and dark soils, located in micro- and inter-hill depressions [12].

As a result of comprehensive studies in the floodplain forests of the Republic of Mari El in the three selected phytocenotic tiers, tree species with a complete ontogenetic structure were noted. Among honey-producing species in floodplain forests, winter linden tree is most common. European white elm (*Ulmus laevis* Pall.) is also fairly common, but with an insignificant presence in the stand composition. Studying the diversity, age, and spatial structure of the stand, scientists noted that smooth elm has the worst life condition [13].

The purpose of the study is to identify honey-producing species in the components of the forest phytocenosis in ravine and floodplain elm groves and to determine the honey productivity of plant formations.

## MATERIAL AND METHODS

For conducting research in ravine elm groves during 2018–2021, trial plots and accounting areas were established in the territory of the Kasharskoe and Shakhtinskoe forestries, and for accounting of floodplain elm groves – in the Bagaevskoe and Morozovskoe forestries of the Rostov region. Trial plots were laid out using materials from forest management enterprises (plans of forest stands, taxation descriptions). The type of forest and topographic location were indicated in accordance with the



**Рис. 1.** Схема лесорастительного районирования бассейна р. Дон

**Fig. 1.** Scheme of the forest zoning of the Don River basin

taxation descriptions [14]. In describing the forest management-taxation characteristics, the following abbreviations were used: ROGLB – ravine oak groves on light banks, ROGGS – ravine oak groves on gully slopes, ROGSB – ravine oak groves on shady banks, EFVG – elm forest of various grasses, GEFVGS – gramineous elm forest of various grasses with sedge, TM – Tatarian maple, E – elm, WW – white willow, GA – green ash, ALM – ash-leaved maple, CP – common pear, BP – black poplar, EE – English elm (plain elm), LSO – low-stemmed oak, FM – field maple, CA – common ash.

The identification of honey plants in the phytocenosis components, conducting a quantitative account of honey plants and their flowering intensity, collecting nectar samples to determine the honey productivity of plant formations were carried out according to the “Methods of conducting research in beekeeping” [14].

## RESULTS AND DISCUSSION

The formation of ravine oak groves occupies over 90% of the area of ravine forests, predominantly located in the northern and central parts of the Rostov region. Elm groves are widespread in the Veshensk district in the floodplain of the middle course of the Don River. As a companion species, elm is found in the floodplains of the Don and Seversky Donets tributaries along with English elm.

Elm groves are favored by moist habitats on rich meadow and meadow-swamp soils. The groundwater level under the studied formations ranges from 0.8 to 1.5 meters. In the southern regions, the condition of the elm worsens, accompanied by drying of the stand.

*European white elm, common, large-leaved (U. laevis* Pall.) grows throughout the Rostov region in ravine mixed forests, at the lower parts of ravine banks as a mixture with the main stand, and as a second tier. It is more commonly found in river floodplains, preferring moist soils. It is a large tree with a slender trunk and branched crown. It is easily distinguished from other species by its bark, which is initially smooth and later forms a gray-brown crust that peels off in thin plates. The flowers on long stalks are gathered in hanging bunches. It blooms in April before the leaves unfold and is a valuable early

honey plant, providing a lot of nectar and pollen. On warm days, bees eagerly visit the flowers of elm, collecting nectar, pollen, and glue from the buds.

*Scotch elm (U. scabra* Mill.) is cold-resistant. It is most common in the north of the region. Scotch elm groves are sporadically noted along the edges, bottom parts of ravines, and as forest islands on riverside slopes. It differs from European white elm by its rough upper leaves with an asymmetric base. It blooms in April and provides a lot of nectar and pollen.

*Cork elm (U. suberosa* Moench.) grows in shrubs, mainly in floodplain oak groves and in the undergrowth of ravine forests. It blooms slightly later than the previous two species and is distinguished from them by branches with wing-like corky outgrowths [6].

*English elm, plain elm (U. campestris* L.) is drought-resistant and a representative of the steppe zone. It is widespread in the southern forestry regions of the Rostov region (Ust-Donetsk, Romanovskiy). It differs from cork elm in its branches, which lack corky outgrowths.

In addition to these forms, the dwarf elm (*U. pumila* L.) is cultivated in gardens and parks. All of them produce a lot of nectar and pollen and are good early spring honey plants. Almost every year, bees collect honeydew from the leaves of elms.

Research has revealed that the taxation indicators of middle-aged elm stands on the banks of light-exposure ravines are higher than those of gully slopes (see Table 1).

In the fresh sub-forest of the Shakhtinskoe forestry, in the mature age group, European white elms reach an average height of 16.0 meters, and the average trunk diameter is 26.0 cm, while in young stands, the figures are 6.3 meters and 8.7 cm, respectively.

English elm, along with high honey-producing field maple, as well as European white elm and low-stemmed oak, is found on the riverside slope of the eastern exposure with a slope steepness of 20 degrees. In the understory, oleaster, a May honey plant, is rarely noted.

In the herbaceous layer, honey plants such as common yarrow, Jerusalem sage, horehound, steppe sage, and eight-leafed bedstraw, which bloom at different times, are found. Among them are honey



**Табл. 1.** Лесоводственно-таксационная характеристика объектов исследования  
**Table 1.** Silvicultural and taxational characterization of the study sites

Forest type	FST (forest site type)	Composition	Forest density	Age, years	Height, m	Diameter, cm
<i>Ravine elm forests</i>						
On light banks	E <sub>0-1</sub>	5E2GA1LSO2 FM	0,5	30	11,0	12,2
		4E2CA2ALM 2TM	0,6	60	14,0	18,1
	E <sub>1</sub>	4LSO4EE2TM	0,8	30	10,0	14,4
		8E1CA1LSO	0,7	20	6,7	10,5
		7EE3LSO	0,7	60	15,0	18,3
On shady banks	E <sub>2</sub>	6E2TM1LSO1GA	0,6	16	6,3	8,7
		8E2WW	0,5	80	16,0	26,0
Gully slopes	E <sub>0-1</sub>	7EE3FM	0,6	50	10,3	14,6
		5B2LSO3EE	0,7	60	12,1	14,1
<i>Floodplain elm forests</i>						
Various grasses	D <sub>2</sub> H <sub>1</sub>	4E4GA2WW + M + ALM	0,6	50	9,0	16,2
		8E2WW+ GA	0,7	50	15,2	22,0
		10E+ WW	0,7	30	10,4	16,3
		5E4WW1BP	0,7	40	12,6	16,7
		6B2WW1ALM1CP	0,4	30	11,0	12,5
		7TM3E	0,8	40	10,4	10,2
Gramineous elm forest of various grasses with sedge	D <sub>2</sub> H <sub>1</sub>	5E3WW2BP + GA	0,5	60	13,6	23,2
		9WW1E	0,4	55	15,2	22,1
		4E3GA3WW + ALM	0,7	40	12,2	14,0

plants with an extended blooming period, forming a continuous honey-producing conveyor.

To study the honey productivity of floodplain forests, we have established trial plots in plantations of interest to beekeeping (see Table 1, Fig. 2).

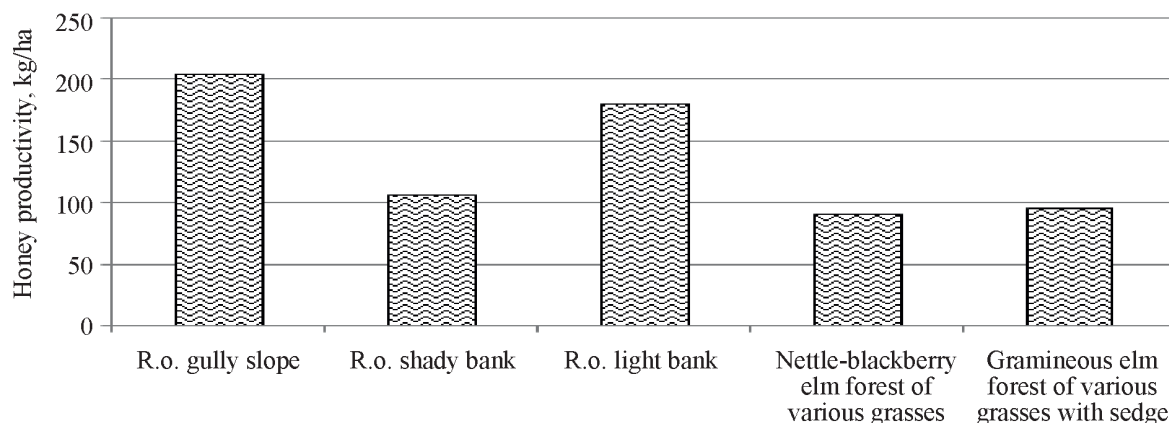
Analyzing the obtained forest management-taxation characteristics (see Table 1), we found that in terms of height, the gramineous elm forest of various grasses with sedge in the Bagaevskoe forestry stands out among middle-aged stands compared to the nettle-blackberry elm forest of various grasses – 13.0 and 12.6 meters, respectively. In terms of diameter, the opposite relationship of values is noted – 14.0 and 16.7 cm. Changes in the composition of the stand have been identified on the studied trial plots. For instance, in the gramineous elm forest of various grasses with sedge, ash and ash-leaf maple are mixed with the main species, while in the nettle-blackberry elm forest of various grasses, the accompanying species are white willow and pollen-bearing black poplar.

Having clarified the silvicultural and taxonomic characteristics of the stand, identifying the presence of valuable species of honey-bearing plants by com-



**Рис. 2.** Вязовник в пойме р. Дон. Багаевское участковое лесничество. Сбор цветков для анализа нектара

**Fig. 2.** Elm tree in the floodplain of the Don River. Bagaevskoye district forestry. Collection of flowers for nectar analysis



**Рис. 3.** Медовая продуктивность формаций вязовников

(Д.б. – дубняки байрачные; В. – вязовник)

**Fig. 3.** Nectariferous productivity of the elm forest formations

(R.o. – ravine oak forest; E. – elm)

ponents of forest phytocenosis, we determined the honey productivity by forest type in ravine elm forests and floodplain forests (see Table 2). It can be concluded that elm forests with insignificant honey productivity (106.4-203.3 kg/ha) are valuable sites for beekeeping.

Let's analyze the components of the honey lands of the studied formations.

On the shady banks of ravine oak forests, European white elm is characterized by the highest honey productivity (32.9 kg/ha) among elms, as it accounts for 60-80% of the stand composition (see Fig. 3). On the gully slope in the studied forest conditions, the plant formation of elms in the stand with field maple improves the total honey productivity up to 203.3 kg/ha. On the light banks, significant honey productivity (42.6 kg/ha) is characterized by honey-bearing underwood consisting of rose hips, sloe, common pear and hawthorn, which are found in forest glades and forest edges [6]. Herbaceous vegetation under the canopy and at the edges of the stand is characterized by adaptation to the conditions of light and soil moisture, species composition, occurrence and intensity of nectar production. Common yarrow and steppe sage respectively play a certain role in the composition of honey-bearing lands with significant honey productivity of 25 and 100 kg/ha on the light banks of ravine elms.

In the nettle-blackberry elm forest of various grasses, European white elm and white willow provide the main honey yield, while in the underwood - hart's-thorn and alder, European cranberry bush

(35.3 kg/ha). In live ground cover, blackberry, bedstraw and bird vetch are characterized by honey productivity (30.3 kg/ha).

## CONCLUSION

Observations of the state of tree stands and the dynamics of forest management-taxation characteristics in different forest-growing conditions allowed us to establish the dependence of honey productivity on these indicators. Due to the presence of elms, the tree tier of this formation is a source of significant honey collection in early spring during the period of strengthening the bee family. Various *Acer* species and representatives of herbaceous vegetation play an important role in the formations of ravine and floodplain elm groves, but their flowering does not coincide with that of the elms, thereby increasing the honey value of *Ulmus* in the absence of flowering honey plants.

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**Табл. 2.** Цветение и медовая продуктивность формаций байрачных и пойменных вязовников по типам леса

**Table 2.** Flowering and honey productivity of formations of the ravine and floodplain elms by forest types

Honey plant type	Phytocoenosis component/ honey bee participation in the forest stand composition	Flowering time	Honey productivity, kg/ha
<i>Ravine oak forests on light banks</i>			
English elm	Forest stand/40–70 40–80	–	26,2
European white elm		08.IV – 14.IV	
Low-stemmed oak	Forest stand	–	35,5
Field maple		25.IV – 09.V	
Tatarian maple		12.V – 26.V	
Hawthorn		07.V – 18.V	
Common pear	Shrub vegetation	–	42,6
Roschip		17.V – 27.V	
Black thorn		19.IV – 28.IV	
Thousand-leaf	Herbage cover	–	70,6
Sage		25.V – 25.VI	
Total			179,3
<i>Ravine oak forests on shady banks</i>			
European white elm	Forest stand /60–80	08. IV –14. IV	32,9
Tatarian maple		12.V –26.V	
Low-stemmed oak	Forest stand	–	12,3
White willow		18.IV –29.IV	
Hawthorn		07.V –18.V	
Common pear		–	
Black thorn	Shrub vegetation	–	22,5
Jerusalem sage		19.IV – 28.IV	
Hoarhound	Herbage cover	–	20,2
Total		–	
<i>Ravine oak forests on gully slopes</i>			
English elm	Forest stand /30–70 50	–	9,5
European white elm		08.IV –14.IV	
Field maple	Forest stand	25.IV – 09.V	151,0
Low-stemmed oak		–	
Oleaster	Shrub vegetation	–	4,8
Sage		25.V –25.VI	
Bedstraw	Herbage cover	–	33,3
Total		–	
<i>Nettle-blackberry elm forest of various grasses</i>			
European white elm	Forest stand /40–50	08.IV –14.IV	50,0
Roschip		17.V –27.V	
Alder buckthorn	Undergrowth	14.V –26.V	35,3
Cranberry tree		14.V – 20.V	
Hart's thorn	Grass cover	–	30,3
Dewberry		–	
Bedstraw		–	
Bird vetch		13.VI – 15.VII	
Total			90,7
<i>Gramineous elm forest of various grasses with sedge</i>			
European white elm	Forest stand /10–50	08.IV – 14.IV	55,7
Alder		14.V – 26.V	
Hart's thorn	Undergrowth	–	40
Total		–	

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#### ИНФОРМАЦИЯ ОБ АВТОРЕ

✉ Самсонова И.Д., доктор биологических наук, доцент, профессор; **адрес для переписки:** Россия, 194021, Санкт-Петербург, Институтский переулок, 5; e-mail: isamsonova18@mail.ru

#### AUTHOR INFORMATION

✉ **Irina D. Samsonova**, Doctor of Science in Biology, Associate Professor, Professor; **address:** 5, Institutsky lane, Saint-Petersburg, 194021, Russia; e-mail: isamsonova18@mail.ru

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## ХАРАКТЕР НАРАСТАНИЯ ГРИБНОЙ ИНФЕКЦИИ В СОРТОВЫХ БИОЦЕНОЗАХ ОЗИМОЙ РЖИ И ПОИСК УСТОЙЧИВЫХ ГЕНОТИПОВ

✉ Шчеклеина Л.М., Шешегова Т.К.

Федеральный аграрный научный центр Северо-Востока им. Н.В. Рудницкого  
Киров, Россия

✉ e-mail: [immunitet@fanc-sv.ru](mailto:immunitet@fanc-sv.ru)

Изучено 26 генотипов озимой ржи селекции ФАНЦ Северо-Востока и 43 образца из коллекции Всероссийского института генетических ресурсов растений им. Н.И. Вавилова (ВИР). Полевые исследования проведены в 2020–2022 гг. в условиях Кировской области на естественном фоне развития грибных болезней с целью поиска новых генетических источников с комплексной неспецифической устойчивостью к грибным болезням. Мониторинг болезней (с фазы 31 по 85 по шкале Zadoks) позволил провести анализ нарастания грибной инфекции и выявить восприимчивые генотипы. При учете болезней использовали общеизвестные методики. Характер растительно-микробных взаимодействий и параметры устойчивости оценивали по показателям ПКРБ (площадь под кривой развития болезни) и ИУ (индекс устойчивости). К восковой спелости только один коллекционный образец Россиянка 2 сохранял высокую устойчивость к септориозу в сочетании с признаком «slow rusting». Показатель ПКРБ составил 105 единиц, ИУ – 0,12. Приведенные уравнения регрессии носят линейный характер, на основании которых можно утверждать, что суточное нарастание болезней по тренду на сортах ФАНЦ Северо-Востока составляет 5,4...9,9% (бурая ржавчина) и 8,4...16,4% (стеблевая); на образцах коллекции ВИР – 6,4...12,1% (бурая) и 15,0...45,0% (стеблевая). Величина коэффициента детерминации  $R^2 = 90-99\%$  характеризуется как сильная. Отмечена более высокая ржавчиноустойчивость сортов ФАНЦ Северо-Востока по сравнению с изученными образцами коллекции ВИР. Выявлено 24 сорта озимой ржи с неспецифической устойчивостью к двум грибным болезням и более и медленным «slow rusting» нарастанием инфекции в течение онтогенеза (Флора, Графиня, Перепел, Графит, Графит ФП, Лика, Гармония, Симфония, Румба крупнозерная, Wibro, Кауро, Pastewne Zielone и др.). Данные сорта могут быть использованы в селекции на повышение фитоиммунитета к конкретным болезням в качестве источников признака.

**Ключевые слова:** *Secale cereale* L., мучнистая роса, септориоз, бурая и стеблевая ржавчина, показатель ПКРБ, индекс устойчивости, источники устойчивости

## THE NATURE OF FUNGAL INFECTION GROWTH IN VARIETAL BIOCOENOSES OF WINTER RYE AND THE SEARCH FOR RESISTANT GENOTYPES

✉ Shchekleina L.M., Sheshegova T.K.

Federal Agrarian Research Center of the North-East named N.V. Rudnitsky  
Kirov, Russia

✉ e-mail: [immunitet@fanc-sv.ru](mailto:immunitet@fanc-sv.ru)

26 genotypes of winter rye from the selection of FASC of the North-East and 43 samples from the collection of the All-Russian Institute of Plant Genetic Resources named after N.I. Vavilov (VIR) were studied. Field studies were conducted in 2020–2022 in the conditions of the Kirov region on the natural background of fungal disease development in order to search for new genetic sources with complex non-specific resistance to fungal diseases. Disease monitoring (phases 31 to 85 on the Zadoks scale) allowed analysis of fungal infection build-up and identification of susceptible genotypes. Commonly known techniques were used to record the diseases. The nature of plant-microbial interactions and resistance parameters were evaluated by the AUDPC (area under disease progress curve) and the RI (resistance index) figures. By wax ripeness, only one collection sample, Rossiyanka 2, retained high resistance to septoriose in combination with the “slow rusting” trait. The AUDPC indicator was 105 units and the RI was 0.12. The given regression equations are linear in nature, on the basis of which it can be stated that daily increase of diseases according to the trend on the varieties of FASC of the North-East is 5.4...9.9% (brown rust) and 8.4...16.4% (stem rust); on the VIR collection samples – 6.4...12.1% (brown rust) and 15.0...45.0% (stem rust). The value of the coefficient of determination  $R^2 = 90-99\%$  is characterized as strong. Higher rust resistance of the FASC of the North-East varieties was noted in comparison with the

studied samples of the VIR collection. 24 winter rye varieties with nonspecific resistance to two or more fungal diseases and slow “slow rusting” increase of infection during ontogenesis were identified (Flora, Grafinya, Perepel, Grafit, Grafit FP, Lika, Harmony, Symphony, Rumba large-grained, Wibro, Kaupo, Pastewne Zielone, etc.). These varieties can be used in breeding for increasing phytoimmunity to specific diseases as the trait sources.

**Keywords:** *Secale cereale* L., powdery mildew, septoriose, leaf and stem rust, AUDPC index, resistance index, sources of resistance

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Авторы заявляют об отсутствии конфликта интересов.

#### Conflict of interest

The authors declare no conflict of interest.

## INTRODUCTION

Winter rye is a unique grain crop that is not demanding in terms of growing conditions: soil fertility, pesticides, and agrochemicals. It has high winter hardiness, frost resistance, tolerance to soil drought, and acidity. It is known that commercial plantings of rye slow down the development of weeds, preserve the soil cover from destruction, and improve its structure [1–5]. Currently, the sown area under winter rye is less than 1 million hectares. The main plantings are located in the Volga Federal District, where 74% of all rye areas are concentrated (in 2020 – 730.9 thousand hectares), of which 58 thousand hectares are in the Kirov region [6]. The prolonged vegetative period and instability of climatic factors in the Kirov region exacerbate the phytosanitary situation in winter rye crops, creating a suitable environment for various infections such as snow mold, root rots, powdery mildew, rust, ergot, etc. [1, 7].

In Russia, there are still no varieties of winter rye with resistance at the threshold of harmfulness level. Long-term protection against mass infection of plants with infectious diseases over a large territory will increase grain production in specific agroclimatic conditions, as well as im-

prove its quality and control in crops. Therefore, one of the important tasks of breeding improvement of this crop is the identification of effective gene sources of resistance for breeding [8, 10]. Currently, research in breeding for immunity involves the search for genotypes with long-term nonspecific resistance, which manifests as a reduction in the number of pustules, scabs, spots on the surface of the affected organ, an increase in the duration of the latent period of pathogenesis, and a decrease in the rate of development of epiphytoty. For winter rye, an important food crop in conditions of constant presence of disease pathogens and long-term variety rotation, resistance to the slow buildup of fungal diseases<sup>1</sup> is characteristic. Disease development forecasting allows monitoring their pathogenicity and early disease development and identifying genotypes with slow infection accumulation “slow rusting”<sup>2</sup>.

Additionally, in analyzing plant-microbial interactions and accounting for diseases, two indicators of resistance are used: AUDPC (area under the disease progress curve) and RI (resistance index).

The purpose of the research is to search for new genetic sources with complex nonspecific

<sup>1</sup>Zhuchenko A.A. Adaptive plant growing (ecological and genetic bases) theory and practice. M.: Agrorus publishing house, 2009, vol. 3, 958 p.

<sup>2</sup>Kolomiets T.M., Kovalenko E.D., Pankratova L.F., Skatenok O.O., Bockelman H. Study of partial resistance parameters in wheat varieties to the pathogens *Stagonospora nodorum* and *Septoria tritici* // Immunological protection of agricultural crops from diseases: theory and practice: Proceedings of the International Scientific and Practical Conference dedicated to the 125th anniversary of the birth of N.I. Vavilov. Bolshiye Vyazmy, Moscow region, 2012, pp. 257–261.

resistance to fungal diseases among domestic varieties of winter rye and samples from the VIR collection.

Research objectives:

– study of different gene pool materials of winter rye in terms of the development of epiphytotically dangerous fungal diseases under natural infectious load of pathogens;

– identification of the least diseased varieties and samples, as well as forms with slow infection build-up in biocoenoses for phyto immunity breeding.

## MATERIAL AND METHODS

The research was conducted at the Federal Agrarian Scientific Center of the North-East (FASC Northeast) in 2020-2022. Field studies were carried out at the experimental field of FASC North-East (Kirov city) following a fallow forecrop. Nitroammophoska was applied at 1.5 c/ha before pre-sowing cultivation. The agricultural technology was the standard practice for the central zone of the Kirov region. The soil of the experimental plots is sod-podzolic, medium loamy. The agrochemical characteristics of the soil in the nurseries: humus content – 2.43-3.56%; mobile phosphorus content – 334-349 mg/kg; exchangeable potassium – 232-304 mg/kg of soil; pH of the salt extract – 5.0-5.4.

The research material comprised 26 promising varieties from the selection of FASC Northeast and 43 samples from the VIR collection (St. Petersburg). The studied material was sown in the collection nursery and competitive variety testing department of winter rye at FASC Northeast. Plot sizes were 1 and 15 m<sup>2</sup>, with two and six repetitions, respectively. Field experiments were laid out according to the methodology of state variety testing<sup>3</sup>. Diagnosis and registration of disease development were determined by

standard methods<sup>4,5</sup>. The degree of development was assessed 3-5 times during the plant development period – from the boot stage to the wax ripeness of the grain (phases 31-85 according to the Zadoks scale) at intervals of 10-16 days from the first symptoms of damage to the cessation of the pathological process.

The AUDPC (Area Under the Disease Progress Curve) is calculated using the formula proposed by D.F. Johnson and R.D. Wilcoxson<sup>6</sup>. Absolute AUDPC values vary annually depending on agrometeorological conditions and infectious load, with an additional criterion being the RI (Resistance Index). The RI classifies genotypes by the level of partial resistance or “slow rusting”. The RI is calculated using a formula<sup>7</sup> and divided into four groups: 0.10...0.35 – high resistance level; 0.36...0.65 – medium; 0.66...0.80 – low; more than 0.81 – susceptible.

Statistical processing was performed using the AGROS software package for statistical and biometric-genetic analysis in plant breeding and selection (version 2.07.) and Microsoft Office Excel.

## RESULTS AND DISCUSSION

To identify the genotypes with different types of nonspecific disease resistance (with a prolonged incubation period and slow infection build-up), collection samples and varieties from FASC Northeast were evaluated in the dynamics of disease development. Accounts were conducted from the first symptoms of damage. Such monitoring allows forecasting early development of epiphytoties and identifying genotypes susceptible at the beginning of ontogenesis, as well as forms with slow infection build-up. These studies are particularly relevant for crops with long variety rotation, such as winter rye (see footnote 1).

<sup>3</sup>Methodology of state variety testing of agricultural crops. Moscow, 1985, Issue 2, P. 2, 230 p.

<sup>4</sup>*Kobylansky V.D., Koroleva L.A.* Methodical guidelines for selection of winter rye for resistance to fungal diseases. Leningrad, 1977, 26 p.

<sup>5</sup>*Sheshegova T.K., Kedrova L.I.* Methodical recommendations on creation of artificial infection backgrounds and evaluation of winter rye for resistance to diseases. Kirov, 2003, 30 p.

<sup>6</sup>*Johnson D.F., Wilcoxson R.D.* A table of areas under disease progress curves. Technical Bulletin, Texas Agriculture Experiment Station. Texas, 1981, N 1377, pp. 2–10.

<sup>7</sup>*Makarov A.A., Strizhekozin Y.A., Solomatin D.A.* Quantitative classification of wheat varieties by the degree of race-specific resistance to brown rust // Immunity of agricultural crops to pathogens of fungal diseases. Moscow, 1991, pp. 105–110.



There were no critical deviations in temperature and water regimes. The winter vegetation of plants occurred under a thick snow cover. Favorable water and thermal regimes in the spring contributed to the active regeneration of winter rye. Due to the cold conditions in May (average temperature below normal by 3.4 °C), there was a delay in plant ontogenesis at the beginning of vegetation and the development of phytopathogenic fungi. Therefore, sufficient soil moisture and a relatively favorable temperature background in the summer period of rye vegetation contributed to the development of fungal diseases, allowing an objective assessment of the winter rye gene pool material.

Interaction: *Secale cereale* – *Blumeria graminis*. The most severe development of powdery mildew infection reduces plant productivity, decreases leaf assimilation of nutrients, and destroys chlorophyll [10, 11]. According to our data, the disease occurs in the Kirov region with a frequency of 4-5 times in 10 years, with harmfulness of 10-15%<sup>8</sup>. The first signs of *B. graminis* among the studied material were noted in the phase of the beginning of heading (phase 51 according to the Zadoks scale). Seven varieties from FASC Northeast selection (Kirovskaya 89, Flora, Harmony, Symphony, Perepel, Lika, and Graphite FP) and seven collection samples (Kaupo (Latvia), Maru 4 (Japan), SCW 1662, Borulles, Danae (Germany), and two samples from the V.D. Kobylansky collection) showed no symptoms of powdery mildew. These genotypes are presumed to have a more extended incubation period. The most severe development of the disease was observed in the third and fourth registrations (phase 69–75) in four varieties: NVAK 285/15 (28.3%), Nioba (28.0), Nika 3 NP (40.0%), and Folud (40.0%). Resistance (disease development up to 15.0%) was found in six varieties from FASC Northeast (Flora, Grafinya, Lika, Harmony, Symphony, Perepel) and eight collection samples (IN-14, Wiedmannsdank, SCW 1662, Borulles, Danae (Germany), Low-stemmed (Bulgaria), and two samples from the V.D. Kobylansky collection). Judging by the

relatively low AUDPC values (233...370 and 225...363 units, respectively), there is relatively slow build-up of powdery mildew infection in the biocenoses of these genotypes. The susceptibility index of most studied varieties was noted at 0.36...0.65, indicating a medium level of resistance to powdery mildew. Exceptions are the varieties Borulles, Danae, SCW 1662, and samples from the V.D. Kobylansky collection, with a degree of infestation up to 15.0%, and RI – 0.26...0.29. In indicator varieties, the values of AUDPC = 634 units (Kiprez) and AUDPC = 875 units (Nika 3 NP).

Interaction: *Secale cereale* – *Septoria nodorum*. Over 10 years of observation, the occurrence of Septoria infection in the region on rye crops appeared 3–4 times with harmfulness of 10–15% (see footnote 8). Since 2012, the frequency and development of the disease have intensified, and almost every year, more than half of the rye areas are affected by this disease [7]. During the study of winter rye's gene pool, weak development of Septoria from 0.5 to 10.0% was diagnosed in phases 51–59. Symptoms were absent in four FASC Northeast selection varieties (Kirovskaya 89, Grafinya, Harmony, Symphony) and five collection samples (Polko (South Africa), Wibro (Poland), Kaupo (Latvia), Rossiyanka 2, and Falenskaya Universal NP), indicating a more prolonged latent period in the pathosystem. At the beginning of milk ripeness, all FASC Northeast varieties still maintained high resistance; 18 collection samples retained this trait. Intensive Septoria build-up occurred during the wax ripeness phase. The most resistant (disease development up to 15.0%) and characterized by “slow rusting” features were FASC Northeast varieties Harmony and Perepel, and collection samples: Polko (South Africa), Wibro (Poland), Kaupo (Latvia), Pastewne Zielone (Poland), Carsten's (Germany), Talovskaya 2, Rossiyanka 2, Falenskaya Universal, Ottawa NP, and a sample from the V.D. Kobylansky collection. Only one collection sample, Rossiyanka 2, maintained high resistance to the disease combined with the “slow rusting” trait. AUDPC was 105

<sup>8</sup>Kedrova L.I. Winter rye in the North-Eastern region of Russia. Kirov: NIISKh Severo-Vostoka. 2000, 158 p.

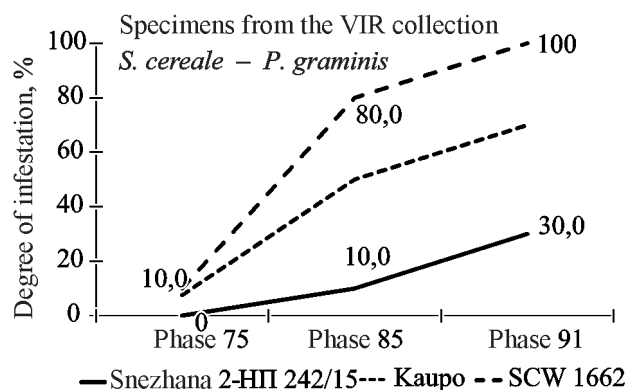
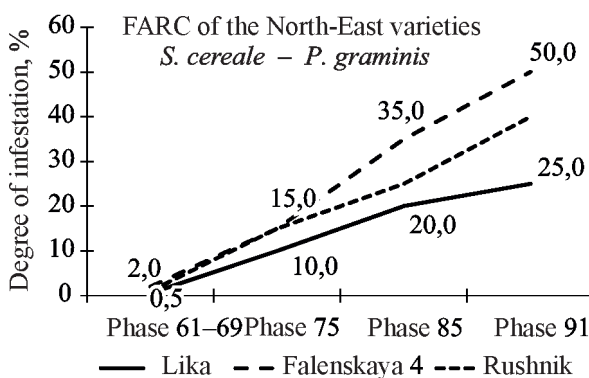
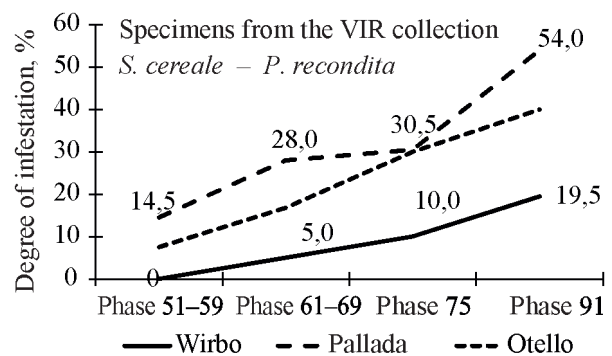
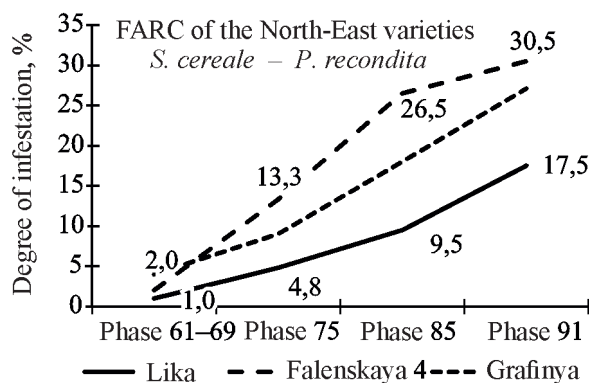
units, RI – 0.12, compared to indicator varieties with AUDPC values of 527 units (Rosa) and 870 units (Borulles).

Interaction: *Secale cereale* – *Puccinia recondita*. According to our data, rust infection in the Kirov region occurs on average 5–7 times (brown) and 3–4 times (stem) with harmfulness levels of 10–15% and 20–50% respectively (see footnote 8). The first pustules of *P. recondita* on leaves were detected in phases 51–55 (see figure). Disease symptoms were absent in eight FASC Northeast varieties (Kirovskaya 89, Snezhana, Flora, Grafinya, Lika, Symphony, Rumba Large Grain, Sarmat) and six collection samples (Wirbo (Poland), Kaupo (Latvia), Pastewne Zielone (Poland), IN-14, Wiedmannsdank (Germany), and Snezhana 2 NP 242/1). The intensification of rust infection up to 54.0% occurred by phase 75. AUDPC was 874 (Snezhana) and 1245 units (Palada); SI – 0.98 and 0.93. In these conditions, moderate resistance (disease development up to 20.0%) was noted in FASC

Northeast varieties (Rumba large-grained, Flora, Graphite, and Graphite FP) and VIR collection samples (Wirbo, Kaupo, and Pastewne Zielone). AUDPC values were 367...427 and 275...366, RI – 0.42...0.52 and 0.22...0.35.

Interaction: *Secale cereale* – *Puccinia dispersa*. Increasing severity of rye stem rust infection leads to reduced grain weight per spike, 1000 grain weight, number of grains per spike, and grain filling, and infected stems become more prone to lodging [12]. The first symptoms of stem rust appeared during the grain-filling period (phase 71–75). In most collection samples and FASC Northeast varieties, no pathogen pustules were detected. The development of rust infection increased with rising temperatures from +18.6 °C (first ten-day period of July) to +20.2 °C (second and third ten-day periods of July), as evidenced by the level of infection in the studied varieties at subsequent accounts.

Disease development in indicator varieties across different gene pool material reached



Нарастание инфекции в биоценозах сортов, отличающихся уровнем горизонтальной устойчивости к видам ржавчины

The growth of infection in the biocenoses of the varieties that differ in the level of horizontal resistance to rust species

50.0% (NVAK 285/15) and 100% (Otello, Edelhofer New, Coro Kurz, and SCW 1662), characterizing the natural infection background as very harsh (see the figure). Against this background, among the FASC Northeast varieties, the least affected (up to 15.0%) are Lika, Harmony, Symphony, and Graphite, with AUDPC values of 135...203 units. In the VIR collection, the least infection (up to 30.0%) was found in two samples from the V.D. Kobylansky collection, as

well as in the varieties Snezhana 2 NP 242/15, Donskaya NP, and Nika 3 NP.

Thus, in the studied gene pool material of winter rye, genotypes have been identified that exhibit resistance to two or more fungal diseases and can be used in breeding to increase resistance to these diseases as sources (see Table 1).

For a part of the studied gene pool, which varies in the level of rust infection, the trend of increasing rust infection was analyzed. The

**Табл. 1.** Источники комплексной устойчивости и медленного нарастания грибной инфекции

**Table 1.** Sources of complex resistance and slow growth of fungal infection

Powdery mildew	Septoriose	Brown rust	Stem rust
Flora, Grafinya, Lika, Harmony, Symphony, Perepel, IN-14, Wiedmannsdank, SCW 1662, Borulles, Danae, Short stature variety, two samples from the V.D. Kobylansky collection	Harmony, Perepel, Polko, Wibro, Kaupo, Pastewne Zielone, Carsten's, Talovskaya 2, Rossiyanka 2, Falenskaya Universal, Ottawa NP, two samples from the V.D. Kobylansky collection	Grafit, Rumba large-grained, Flora, Grafit FP; Wirbo, Kaupo, Pastewne Zielone	Grafit, Rumba large-grained, Flora, Grafit FP, Wirbo, Kaupo, Pastewne Zielone
<i>Degree of infestation of resistant varieties, %</i>			
11,7...15,5	10,0...15,0	16,3...20,5	10,0...30,0
<i>AUDPC indicator</i>			
225...380	105...324	275...367	135...277
<i>RI value</i>			
0,26...0,36	0,12...0,37	0,22...0,35	0,13...0,31
<i>Degree of damage in indicator (susceptible) varieties, %</i>			
28,3...40,0	28,3...45,0	42,5...54,0	50,0...100
<i>AUDPC indicator</i>			
634...875	527...870	874...1245	645...923

**Табл. 2.** Уравнения регрессии на различных по восприимчивости к листовостебельной ржавчинной инфекции генотипах ржи

**Table 2.** Regression equations for rye genotypes with different susceptibility to leaf rust infection

Variety, sample	Gene pool material	Regression equation	Coefficient of determination, R <sup>2</sup>
<i>Brown rust</i>			
Lika	FARC of the North-East varieties	$y = 5,42x - 5,35$	0,969
Falenskaya 4		$y = 9,87x - 6,6$	0,962
Grafinya		$y = 7,83x - 5,05$	0,984
Wibro		$y = 6,35x - 7,25$	0,971
Pallada		Specimens from the VIR collection	$y = 12,1x + 1,5$
Otello	$y = 11,07x - 4,1$		0,996
<i>Stem rust</i>			
Lika	FARC of the North-East varieties	$y = 8,35x - 7,0$	0,982
Falenskaya 4		$y = 16,4x - 15,5$	0,994
Rushnik		$y = 12,7x - 11,5$	0,995
Snezhana 2 NP-242/15	Specimens from the VIR collection	$y = 15,0x - 16,67$	0,964
Kaupo		$y = 31,5x - 20,0$	0,959
SCW 1662		$y = 45,0x - 26,67$	0,907

presented regression equations are linear, from which it can be asserted that the daily increase in diseases according to the trend on FASC Northeast varieties is 5.4...9.9% (brown rust) and 8.4...16.4% (stem); in VIR collection samples – 6.4...12.1% (brown) and 15.0...45.0% (stem). The value of the coefficient of determination  $R^2 = 90\text{--}99\%$  is characterized as strong (see Table 2). These data indicate the relatively successful work of FASC Northeast in increasing the rust resistance of winter rye compared to the studied VIR collection samples.

## CONCLUSION

As a result of immunological research, 24 varieties of winter rye were identified, characterized by non-specific resistance to two or more fungal diseases and slow “slow rusting” increase in infection during ontogenesis, among them: Flora, Grafinya, Perepel, Graphite, Graphite FP, Lika, Harmony, Symphony, Rumba large-grained, Wibro, Кауро, Pastewne Zielone, etc. They can be used in breeding to enhance phytoimmunity to specific diseases as sources of the trait.

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#### ИНФОРМАЦИЯ ОБ АВТОРАХ

✉ **Щеклеина Л.М.**, кандидат сельскохозяйственных наук, старший научный сотрудник лаборатории иммунитета и защиты растений; **адрес для переписки:** Россия, 610007, Киров, ул. Ленина, 166-а; e-mail:immunitet@fanc-sv.ru

**Шешегова Т.К.**, доктор биологических наук, ведущий научный сотрудник, заведующая лабораторией иммунитета и защиты растений

#### AUTHOR INFORMATION

✉ **Lucia M. Shchekleina**, Candidate of Science in Agriculture, Senior Researcher of the Laboratory of Immunity and Plant Protection; **address:** 166-a, Lenina St., Kirov, 610007, Russia; e-mail: immunitet@fanc-sv.ru

**Tatyana K. Sheshegova**, Doctor of Science in Biology, Lead Researcher, Head of the Laboratory of Immunity and Plant Protection

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## ОЦЕНКА ГИБРИДОВ ТИМОФЕЕВКИ ЛУГОВОЙ (*PHLEUM PRATENSE* L.) НА СЕМЕННУЮ ПРОДУКТИВНОСТЬ

Клочкова Н.Л.<sup>1</sup>, (✉) Теличко О.Н.<sup>2</sup>

<sup>1</sup>Федеральный научный центр агробиотехнологий Дальнего Востока им. А.К. Чайки  
Приморский край, Уссурийск, Россия

<sup>2</sup>Дальневосточный научно-исследовательский институт защиты растений –  
филиал Федерального научного центра агробиотехнологий Дальнего Востока им. А.К. Чайки  
Приморский край, с. Камень-Рыболов, Россия

(✉) e-mail: biometod@rambler.ru

Тимофеевка луговая – ценная кормовая культура, используемая для создания сенокосов и пастбищ. Представлены результаты исследований семенной продуктивности гибридных образцов тимофеевки луговой. Цель исследования – создать новый гибридный материал для селекции тимофеевки луговой, адаптированный к условиям степной зоны Приморского края. Объектом исследований стали гибриды тимофеевки луговой второго поколения ( $F_2$ ). В селекционном питомнике ( $F_2$ ) третьего года жизни изучено 39 гибридных образцов. Среди них наиболее перспективными оказались следующие: № 4 (Нарымская ♀ × Приморская местная ♂), № 12 (Приморская местная ♀ × Моршанская 69 ♂), № 27 (Приморская местная ♀ × Нарымская ♂) и № 36 (Моршанская 69 ♀ × Приморская местная ♂). По числу генеративных побегов выделились образцы № 12 (Приморская местная ♀ × Моршанская 69 ♂) и № 36 (Моршанская 69 ♀ × Приморская местная ♂) – 19–21 шт./растение. Продуктивность семян у изученных гибридных образцов варьировала от 3,95 г/растение (№ 4, Нарымская ♀ × Приморская местная ♂) до 10,0 г/растение (№ 36, Моршанская 69 ♀ × Приморская местная ♂). Среди них наибольшей продуктивностью характеризовались образцы № 27 (Приморская местная ♀ × Нарымская ♂) – 8,15 г/растение и № 36 (Моршанская 69 ♀ × Приморская местная ♂) – 10,0 г/растение, превышение над стандартом составило 7,2–31,6%. Анализ посевных качеств показал, что наиболее крупносемянным является гибрид № 27 (Приморская местная ♀ × Нарымская ♂), который превзошел стандарт по массе 1000 семян на 0,18 г. По комплексу хозяйственно ценных признаков выделился гибрид № 27 (Приморская местная ♀ × Нарымская ♂).

**Ключевые слова:** гибрид, тимофеевка луговая, продуктивность, побеги, семена, посевные качества

## EVALUATION OF TIMOTHY HYBRIDS (*PHLEUM PRATENSE* L.) FOR SEED PRODUCTIVITY

Klochkova N.L.<sup>1</sup>, (✉) Telichko O.N.<sup>2</sup>

<sup>1</sup>Federal Scientific Center of Agricultural Biotechnology of the Far East named after A.K. Chaiki  
Ussuriysk, Primorsky Territory, Russia

<sup>2</sup>Far Eastern Scientific Research Institute of Plant Protection – Branch of the Federal Scientific Center of Agricultural Biotechnology of the Far East named after A.K. Chaiki  
Kamen-Rybolov, Primorsky Territory, Russia

(✉) E-mail: biometod@rambler.ru

Timothy grass is a valuable forage crop used to create hayfields and pastures. The results of research on seed productivity of timothy grass hybrid samples are presented. The purpose of the research is to create a new hybrid material for selection of timothy grass adapted to the conditions of the steppe zone of the Primorsky Territory. The second generation ( $F_2$ ) hybrids of timothy grass were the object of research. Thirty-nine hybrid accessions were studied in the breeding nursery ( $F_2$ ) of the third year of life. The following accessions were found to be the most promising: No. 4 (Narymskaya ♀ × Primorskaya mestnaya ♂), No. 12 (Primorskaya mestnaya ♀ × Morshanskaya 69 ♂), No. 27 (Primorskaya mestnaya ♀ × Narymskaya ♂), and No. 36 (Morshanskaya 69 ♀ × Primorskaya mestnaya ♂). Accessions No. 12 (Primorskaya mestnaya ♀ × Morshanskaya 69 ♂) and No. 36 (Morshanskaya 69 ♀ × Primorskaya mestnaya ♂) had the highest number of generative shoots (19–21 shoots per plant). The

seeds productivity of the studied hybrids ranged from 3.95 (No. 4, Narymskaya♀ × Primorskaya mestnaya♂) to 10.0 (No. 36, Morshanskaya 69♀ × Primorskaya mestnaya♂) g/plant. Accessions No. 27 (Primorskaya mestnaya♀ × Narymskaya♂) and No. 36 (Morshanskaya 69♀ × Primorskaya mestnaya♂) were characterized by the highest productivity (8.15 and 10.0 g/plant, respectively) exceeding the standard by 7.2–31.6%. Analysis of the sowing characteristics showed that hybrid No. 27 (Primorskaya mestnaya♀ × Narymskaya♂) was the largest seeded hybrid; the 1000 kernel weight was by 0.18 g higher than the standard. Hybrid No. 27 (Primorskaya mestnaya♀ × Narymskaya♂) stood out for its complex of economically important traits.

**Keywords:** hybrid, timothy, productivity, shoots, seeds, sowing characteristics

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**Conflict of interest**

The authors declare no conflict of interest.

## INTRODUCTION

For the successful development of the livestock industry and the improvement of the quality of the products obtained, special attention must be paid to the full-feeding of farm animals [1, 2]. Annual and perennial grasses play an important role in creating a fodder base in the conditions of the Far East [3–5]. Among the grasses, Timothy grass is of particular interest, as this crop is distinguished by its high winter hardiness, nutritional value, and productive longevity [6, 7]. Its use as a grass component in creating pastures and hayfields allows for the production of high-nutritional and balanced feed [8–10].

The assortment of perennial herbaceous crops is insufficient and does not fully meet the requirements of modern agricultural production in terms of fodder and seed productivity. The creation of highly productive grass stands is possible only with the use of high-yielding and adaptive varieties to specific cultivation conditions [11, 12]. In this regard, the role of biogeocenotic selection in creating varieties capable of more fully utilizing the bioclimatic potential of a particular region is increasing.

Moreover, the bred varieties should be characterized by increased energy and protein nutritional value [13–15].

Currently, in the Far Eastern region, only 15 varieties of Timothy grass are approved for cultivation<sup>1</sup>. Therefore, to update the assortment, there is a need to create new high-quality varieties of Timothy grass that meet the requirements of modern forage production.

The purpose of the study is to create new hybrid material for the breeding of Timothy grass, adapted to the conditions of the steppe zone of the Primorsky Territory.

Objectives of the study are:

- to identify hybrid forms with increased seed productivity;
- to determine the sowing qualities of Timothy grass hybrid seeds.

## MATERIAL AND METHODS

Selection nurseries were established in 2020 on the fields of the forage production department of the Federal Scientific Center of Agricultural Biotechnology of the Far East named after A.K. Chaiki following the methodology of B.A. Dospekhov<sup>2</sup>.

<sup>1</sup>State Register of Breeding Achievements Allowed for Use. Moscow: Kolos, 2022, 719 p.

<sup>2</sup>Dospekhov B.A. Methodology of field experiment (with the basics of statistical processing of research results). Moscow: Alliance, 2014, pp. 69–71.

The material for the study consisted of four second-generation hybrids of Timothy grass, obtained through limited-free pollination between parental forms in a polycross nursery on the isolated plots.

The selection nursery was planted in a single-row method. The length of the row was 4.5 m, with a distance of 0.45 m between the rows. Each row contained 20 plants of Timothy grass. Before flowering, the plants were covered with isolators (see Fig. 1). The standard used was the local Primorskaya variety (Primorsky Territory).

The study of the initial material in the selection nursery was conducted using the methods developed at the Federal Williams Research Center of Forage Production and Agroecology<sup>3,4</sup>. Seed productivity was determined after threshing, drying, and cleaning the seeds. Timothy grass plants were harvested by hand as each hybrid ripened.

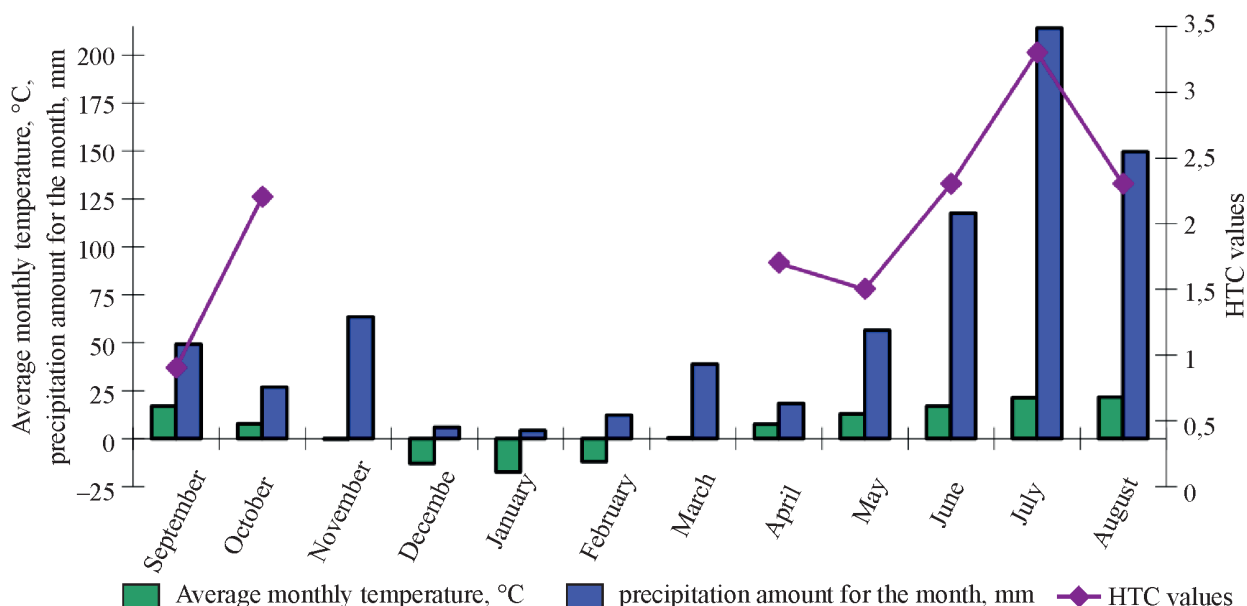
The soil of the experimental plot was meadow-brown podzolic belonging to the heavy loam category by mechanical composition. The humus content was 5.4%.



**Рис. 1.** Гибридный питомник ( $F_2$ ) тимфеевки луговой (2022 г.)

**Fig. 1.** Timothy grass breeding nursery ( $F_2$ ) (2022)

Fig. 2 shows the temperature regime and precipitation distribution by month for 2021 and 2022 (September - December 2021, January - August 2022). The sum of active temperatures per month during the vegetative period of Timothy grass varied from 110 (April 2022) to 649°C (August 2022), and the amount of



**Рис. 2.** Метеорологические условия (2021, 2022 гг.)

**Fig. 2.** Meteorological conditions (2021, 2022)

<sup>3</sup>Kosolapov V.M., Kostenko S.I., Pilipko S.V., Klochkova V.S. Methodological guidelines for breeding perennial grasses. Moscow: All-Russian Research Institute of Forages; Russian State Academy of Sciences; Moscow Agricultural Academy, 2012, 52 p.

<sup>4</sup>Konstantinova A.M., Voshchinin P.A., Novoselova A.S. Methodology of perennial grasses selection. Moscow: All-Russian Research Institute of Forage Crops, 1969, 110 p.



precipitation ranged from 26.7 mm (October 2021) to 214 mm (July 2022).

According to the hydrothermal coefficient, May was characterized as moderately wet (HTC = 1.5), April as wet (HTC = 1.7), October, and June - August as excessively wet (HTC = 2.2–3.3), September as dry (HTC = 0.9).

## RESULTS AND DISCUSSION

In the third-year selection nursery ( $F_2$ ), 39 hybrid samples of Timothy grass were studied. Among them, the following hybrids are considered most promising: No. 4 (Narymskaya ♀ × Primorskaya mestnaya ♂), No. 12 (Primorskaya mestnaya ♀ × Morshanskaya 69 ♂), No. 27 (Primorskaya mestnaya ♀ × Narymskaya ♂), and No. 36 (Morshanskaya 69 ♀ × Primorskaya mestnaya ♂).

The spring aftergrowing of Timothy grass samples was noted on April 12-14, with tube formation beginning from May 17 (No. 27, Primorskaya mestnaya ♀ × Narymskaya ♂) to May 23 (No. 12, Primorskaya mestnaya ♀ × Morshanskaya 69 ♂), and heading from June 13 (No. 27, Primorskaya mestnaya ♀ × Narymskaya ♂) to June 18 (No. 4, Narymskaya ♀ × Primorskaya mestnaya ♂). The vegetative period of the studied hybrids was 127-130 days (see Table 1). The earliest maturing

was the standard (Primorskaya mestnaya) with a vegetative period of 126 days, and the latest maturing was hybrid sample No. 12 (130 days).

The analysis of the change in the height of Timothy grass plants showed that at the tube formation stage, the height varied depending on the sample from 22.0 cm (No. 36, Morshanskaya 69 ♀ × Primorskaya mestnaya ♂) to 29.3 cm (No. 27, Primorskaya mestnaya ♀ × Narymskaya ♂). At the harvest maturity stage, the shortest plants were found in sample No. 36 (Morshanskaya 69 ♀ × Primorskaya mestnaya ♂) and the standard sample (88.4 and 88.6 cm, respectively). The other hybrid samples exceeded the standard in height by 4.7–8.6 cm.

The number of generative shoots is one of the important indicators of the seed yield. Samples No. 12 (Primorskaya mestnaya ♀ × Morshanskaya 69 ♂) and No. 36 (Morshanskaya 69 ♀ × Primorskaya mestnaya ♂), forming 19 and 21 generative shoots respectively, stood out in this regard (see Table 1). Regarding the number of vegetative shoots, hybrid samples No. 36 (Morshanskaya 69 ♀ × Primorskaya mestnaya ♂) – 94 shoots/plant and No. 4 (Narymskaya ♀ × Primorskaya mestnaya ♂) – 90 shoots/plant, had an advantage over the standard. Among the studied hybrids, sample No. 12 (Primorskaya mestnaya ♀ × Morshanskaya 69 ♂) had a low

**Табл. 1.** Период вегетации и морфологические показатели селекционных образцов тимофеевки луговой ( $F_2$ ) (посев 2020 г.), 2022 г.

**Table 1.** Growing period and morphological traits of the studied timothy accessions ( $F_2$ ) (planted in 2020), 2022.

Hybrid number	Hybrid	Vegetation period, days	Generative shoots height before harvesting, cm	Number of vegetative/generative shoots, pcs/plant
4	Narymskaya ♀ × Primorskaya mestnaya ♂	129	95,0	90/14
12	Primorskaya mestnaya ♀ × Morshanskaya 69 ♂	130	93,3	62/19
27	Primorskaya mestnaya ♀ × Narymskaya ♂	127	97,2	88/15
36	Morshanskaya 69 ♀ × Primorskaya mestnaya ♂	127	88,4	94/21
St.	Primorskaya mestnaya	126	88,6	86/17
	LSD <sub>05</sub>		5,1	1,9

count. It is noteworthy that the highest total number of vegetative and generative shoots was formed by hybrid sample No. 36 (Morshanskaya 69♀ × Primorskaya mestnaya♂) – 115 shoots/plant, and the lowest – No. 12 (Primorskaya mestnaya♀ × Morshanskaya 69♂) – 81 shoots/plant. The total number of shoots in hybrid No. 27 (Primorskaya mestnaya♀ × Narymskaya♂) was on par with the standard sample – 103 shoots/plant.

Seed productivity is an important economically valuable trait for any agricultural crop. The seed productivity of the studied hybrid samples of Timothy grass varied from 3.95 g/plant (No. 4, Narymskaya♀ × Primorskaya mestnaya♂) to 10.0 g/plant (No. 36, Morshanskaya 69♀ × Primorskaya mestnaya♂). Among them, samples No. 27 (Primorskaya mestnaya♀ × Narymskaya♂) – 8.15 g/plant and No. 36 (Morshanskaya 69♀ × Primorskaya mestnaya♂) – 10.0 g/plant were the most productive, exceeding the standard by 7.2–31.6% (see Fig. 3).

The seed yield significantly depends on the weight of 1000 seeds. This indicator varied among the samples from 0.35 to 0.53 g. Analysis of the sowing qualities showed that the hybrid No. 27 (Primorskaya mestnaya♀ × Narymskaya♂) was the largest in seed size, surpassing the standard by 0.18 g in the weight of 1000 seeds (see Table 2).

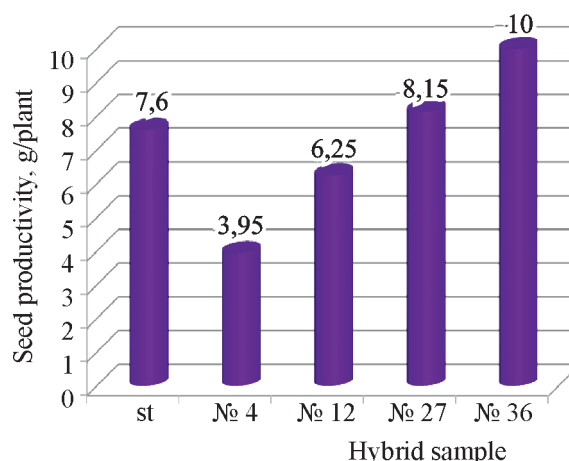


Рис. 3. Продуктивность семян гибридных образцов тимфеетки луговой

Fig. 3. Seed productivity of the studied timothy hybrid accessions

Germination significantly affects the quality of the obtained seeds. According to our data, hybrids No. 4 (Narymskaya♀ × Primorskaya mestnaya♂) – 98%, No. 12 (Primorskaya mestnaya♀ × Morshanskaya 69♂) – 99%, and the standard (Primorskaya mestnaya) – 95% showed good germination. The seed material of all samples met the original seeds and super-elite in terms of germination.

## CONCLUSION

Perennial grasses play an important role in creating hayfields and pastures as they are characterized by longevity, yield of fodder

Табл. 2. Посевные качества семян гибридов тимфеетки луговой ( $F_2$ ) в селекционном питомнике (посев 2021 г.), 2022 гг.

Table 2. Sowing characteristics of the studied timothy hybrids ( $F_2$ ) in the breeding nursery (planted in 2021), 2022

Sample number	Hybrid	Weight of 1000 seeds, g	% to the standard	Germination energy, %	Laboratory germination rate, %
4	Narymskaya ♀ × Primorskaya mestnaya ♂	0,40	114,3	68	98
12	Primorskaya mestnaya ♀ × Morshanskaya 69♂	0,41	117,1	73	99
27	Primorskaya mestnaya ♀ × Narymskaya ♂	0,53	151,4	78	79
36	Morshanskaya 69♀ × Primorskaya mestnaya ♂	0,30	85,7	81	87
St.	Primorskaya mestnaya	0,35	100	96	95
	LSD <sub>05</sub>	0,08			

mass, and high nutritional value. Among the studied hybrids of Timothy grass, samples No. 27 (Primorskaya mestnaya ♀ × Narymskaya ♂) – 8.15 g/plant and No. 36 (Morshanskaya 69 ♀ × Primorskaya mestnaya ♂) – 10.0 g/plant stood out with increased seed productivity. The highest weight of 1000 seeds was characterized by the hybrid sample No. 27 (0.53 g). As a result of the study of hybrid samples of Timothy grass (F2) for a complex of economically valuable traits, hybrid No. 27 (Primorskaya mestnaya ♀ × Narymskaya ♂) was highlighted, which will be used in further breeding work to create a variety.

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## ИНФОРМАЦИЯ ОБ АВТОРАХ

✉ **Теличко О.Н.**, кандидат сельскохозяйственных наук, заведующая отделом; **адрес для переписки:** Россия, 692684, Приморский край, Ханкайский район, с. Камень-Рыболов, ул. Мира, 42а; e-mail: biometod@rambler.ru

**Клочкова Н.Л.**, заведующая лабораторией; e-mail: Klochova128@mail.ru

## AUTHOR INFORMATION

✉ **Olga N. Telichko**, Candidate of Science in Agriculture, Department Head; **address:** 42a, Mira St., Kamen-Rybolov, Khankaisky District, Primorsky Territory, 692684, Russia; e-mail: biometod@rambler.ru

**Natalia L. Klochkova**, Laboratory Head; e-mail: Klochova128@mail.ru

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## ФИЗИОЛОГО-БИОХИМИЧЕСКАЯ ОЦЕНКА УСТОЙЧИВОСТИ СОРТОВ ЯБЛОНИ К ЖАРЕ И ЗАСУХЕ

✉ Киселева Г.К., Ульяновская Е.В., Схаляхо Т.В., Караваяева А.В.

*Северо-Кавказский федеральный научный центр садоводства, виноградарства, виноделия*  
Краснодар, Россия

✉ e-mail: galina-kiseleva-1960@mail.ru

Представлены результаты физиолого-биохимической оценки устойчивости пяти сортов яблони различного эколого-географического происхождения к жаре и засухе в условиях Краснодарского края. Изучены в течение 2020–2022 гг. следующие сорта: Интерпрайс (Америка), Флорина (Франция), Орфей, Марго, Багрянец Кубани (Россия). Показатели водного режима определяли весовым методом после высушивания навесок в термостате при 105 °С до постоянной массы. Содержание фотосинтетических пигментов определяли спектрофотометрически в 85%-й ацетоновой вытяжке. Содержание аскорбиновой кислоты определяли методом капиллярного электрофореза. В условиях напряженности стрессовых факторов летнего периода у сортов Орфей и Марго обнаружено наименьшее снижение оводненности листовых тканей (на 1,32–1,45%) в сравнении с другими изучаемыми сортами (на 2,40–3,27%). У сортов Орфей и Марго выявлены наибольшие показатели отношения связанной воды к свободной во все летние месяцы, которые в августе составляли 9,95 и 9,97 соответственно, у других сортов они составляли 6,54 и 7,46. Стабильное содержание суммы хлорофиллов в течение лета у сортов Орфей и Марго, а также низкие отношения суммы хлорофиллов к каротиноидам, составляющие в августе 2,65 и 2,74 соответственно в сравнении с другими изучаемыми сортами (3,15 и 3,46), свидетельствуют о повышенной адаптивности их листовых тканей. В ответ на засуху у сортов Орфей и Марго установлено увеличение содержания аскорбиновой кислоты в большей степени (в 2,02–2,58 раза) в сравнении с другими изучаемыми сортами (в 1,17–1,59 раза). Сорта Орфей и Марго проявили себя более адаптивными для возделывания в условиях Краснодарского края. Они могут быть использованы в селекции для выведения наиболее устойчивых к жаре и засухе сортов.

**Ключевые слова:** яблоня, адаптивность, оводненность, хлорофилл, каротиноиды, аскорбиновая кислота

## PHYSIOLOGICAL AND BIOCHEMICAL EVALUATION OF RESISTANCE TO HEAT AND DROUGHT IN APPLE VARIETIES

✉ Kiseleva G.K., Ulyanovskaya E.V., Skhalyaho T.V., Karavaeva A.V.

*North Caucasian Federal Scientific Centre of Horticulture, Viticulture, Winemaking*  
Krasnodar, Russia

✉ e-mail: galina-kiseleva-1960@mail.ru

The results of physiological and biochemical evaluation of resistance to heat and drought of five apple tree varieties of different ecological and geographical origin in the conditions of the Krasnodar Territory are presented. The following varieties were studied during 2020–2022: Interprise (America), Florina (France), Orphey, Margo, Bagryanets Kubani (Russia). Indicators of water regime were determined by the weight method after drying the samples in the thermostat at 105°C to constant weight. The content of photosynthetic pigments was determined spectrophotometrically in 85% acetone extract. The ascorbic acid content was determined by capillary electrophoresis. Under the conditions of tension stress factors of the summer period, the varieties Orphey and Margo showed the smallest decrease in water content of the leaf tissues (by 1.32–1.45%) in comparison with the other studied varieties (by 2.40–3.27%). Orphey and Margo varieties showed the highest values of bound water to free water ratio in all summer months, which in August were 9.95 and 9.97, respectively, in other varieties they were 6.54–7.46. Stable content of the sum of chlorophylls during the summer in the varieties Orphey and Margo, as well as low ratios of the sum of chlorophylls to carotenoids, amounting to 2.65 and 2.74 in August, respectively, in comparison with the other studied varieties (3.15–3.46) indicate increased adaptability of their leaf tissues. In response to drought, Orphey and

Margo varieties showed an increase in ascorbic acid content to a greater extent (2.02–2.58 times) compared to the other varieties studied (1.17–1.59 times). Orpheus and Margo varieties have shown themselves to be more adaptable for cultivation in the conditions of the Krasnodar Territory. They can be used in breeding to develop the most heat- and drought-resistant varieties.

**Keywords:** apple tree, adaptability, water content, chlorophyll, carotenoids, ascorbic acid

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**Conflict of interest**

The authors declare no conflict of interest.

## INTRODUCTION

Apple tree is a leading fruit crop cultivated in the Krasnodar region. Soil and climatic conditions of the region are favorable for growing apple varieties with high consumer quality fruits. However, from the 1990s to the 2020s, there has been an increase in the occurrence of extremely high temperatures (above +30 °C) in the spring and summer periods, as well as a decrease in precipitation during critical phenophases for apple trees: bud and flower bud differentiation, fruit set, and growth. These adverse factors ultimately affect the productivity and yield of the plants, highlighting the need for breeding apple trees for adaptability and increased resistance to heat and drought [1].

The apple gene pool of the North Caucasian Federal Scientific Centre of Horticulture, Viticulture, Winemaking (NCFSC HVW) currently comprises 436 samples of various ecological and geographical origins, actively used in the breeding work.

The use of physiological and biochemical indicators to identify highly adaptive varieties of fruit and berry, ornamental, nut and other crops to heat and drought is widely practiced all over the world. Parameters of water exchange, content of photosynthetic pigments, ascorbic acid, anatomo-morphological features of leaf serve as important criteria of drought resistance of plants. Increased water-holding capacity of leaves, increase in the bound form of water and

the regulatory capacity of stomata help to maintain water homeostasis of plants under drought. There is evidence that drought-tolerant varieties are characterized by smaller changes in water content, turgor, sucking power, and pigment composition of leaves [2-4]. It has been shown that drought-resistant apple varieties have more stable chlorophyll content in the leaves during periods of insufficient water availability compared to susceptible ones [5]. In hazelnut leaves, chlorophyll content decreased, and carotenoid content doubled under increased air temperature and lack of precipitation [2]. Significant progress has been made in identifying the genes whose expression increases during water stress and transcription factors involved in regulating response reactions to drought in apple trees [6]. Despite molecular-genetic achievements in the breeding process, physiological-biochemical research remains relevant.

The purpose of the study is to assess the drought and high-temperature resistance of apple varieties of different ecological and geographical origins using physiological-biochemical indicators, to identify varieties with high adaptive resistance for cultivation in the Krasnodar region, and for use in breeding.

## MATERIAL AND METHODS

The research was conducted from 2020 to 2022 at the genetic collection center of the “North Caucasian Federal Scientific Centre of

Horticulture, Viticulture, Winemaking” (NCFSC HVW), located at the Central Agricultural Production Cooperative (Krasnodar city). The research subjects were apple varieties of various ecological and geographical origins – Enterprise (America), Florina (France), Orpheus, Margo, and Bagryanets Kubani (breeds from the North Caucasian Zonal Research Institute of Horticulture and Viticulture, Russia). All varieties were planted on SK2 rootstock in 2013 and arranged in a 4×1.2m planting scheme. The Orpheus variety was used as a control.

Physiologically mature leaves were collected from the middle part of one-year-old shoots (7th to 9th leaf from the base of the shoot) in the middle canopy layer, evenly around its circumference. The studies were conducted in triplicate on ten leaves of each variety. Water regime indicators were determined by the weight method after drying the samples in a thermostat at 105 °C to constant weight. The content of free and bound water in the leaves was determined refractometrically<sup>1</sup>. The content of photosynthetic pigments was determined in an 85% acetone extract using a Unico 2800 spectrophotometer (United Products & Instruments, USA)<sup>2</sup>. The content of ascorbic acid was determined by capillary electrophoresis on a Capel 104R instrument according to the method based on obtaining electropherograms using direct detection of absorbing components of the sample<sup>3</sup>.

Measurements were made in triplicate analytical repetitions. Calculations were performed using Microsoft Excel 2010 software. The least significant difference between the analyzed indicators at the 95% confidence level (LSD0.5) was evaluated, and the arithmetic mean and standard deviation were calculated<sup>4</sup>.

Meteorological conditions varied over the years of the study. In 2020, July was the hottest month, with maximum air temperatures reach-

ing +38.4 °C (4.1 °C above the average multi-year values), and August was the driest month (precipitation – 10.7 mm). In 2021, the maximum air temperature in July reached +37.7 °C (3.4 °C above the average multi-year values), with 28.4 mm of precipitation in July and 75 mm in August. In 2022, the maximum air temperatures in June and July reached +35.3 °C. June was characterized by increased precipitation (161 mm) compared to July and August, with 62.6 and 91.2 mm of precipitation, respectively.

## RESULTS AND DISCUSSION

Water is one of the significant ecological factors ensuring the growth, photosynthesis, mineral nutrition, and other physiological processes in apple trees. Literature pays great attention to apple tree water regime parameters in connection with the issue of resistance to high temperatures and lack of precipitation. The degree of hydration of plant tissues and the relative water content serve as important indicators of plant resistance to drought. According to research by Z.E. Ozherelieva et al. [7], stable leaf hydration in apple trees during the summer indicates greater drought resistance. During vegetation, the water content in apple leaves gradually decreases from 74% at the beginning to 57% at the end.

In our studies, the average leaf tissue hydration of the studied apple varieties in June was 62.95–64.23%, and in July – 61.59–63.53%, as indicated in the table.

In August, there was a further decrease in leaf tissue hydration to 60.55–62.62% in all the studied varieties. It was found that by the end of summer, the leaf hydration decreased the least in the Orpheus (by 1.45%) and Margo (by 1.32%) varieties. The other varieties showed a slight decrease – by 2.40–3.27%.

The ability of plant tissues to retain water is characterized by the state of water, convention-

<sup>1</sup>Kushnirenko M.D., Pecherskaya S.N. Physiology of water exchange and drought resistance of plants. Kishinev: Shtiyintsa, 1991, 306 p.

<sup>2</sup>Gavrilenko V.F., Ladygina M.E., Khandobina L.M. Large workshop on plant physiology. Moscow: Higher Education School, 1975, 392 p.

<sup>3</sup>Yakuba Y.F., Ilyina I.A., Zakharova M.V., Lifar G.V. Methodology for determining the mass concentration of ascorbic, chlorogenic and caffeic acids in shoots and leaves of fruit crops and grapes using capillary electrophoresis // Modern instrumental and analytical methods of research of fruit crops and grapes. Krasnodar: SKZNIISiV, 2015, pp. 68-73.

<sup>4</sup>Dospekhov B.A. Methodology of field experiment (with the basics of statistical processing of research results). 5th edition, revised and supplemented. Moscow: Alliance, 2014, 351 p.

ally divided into free and bound forms. The free form of water is involved only in metabolism, while the bound form of water maintains the water-retaining capacity of the cell and plays a significant role in plant resistance to unfavorable conditions. In summer, the fractional composition of water in the leaves depends on climatic conditions: less precipitation and high air temperature lead to a decrease in the proportion of free water and an increase in the proportion of bound water. A high ratio of bound water to free water indicated high drought resistance in varieties of delicate actinidia and red currant [2, 3].

In our research, the content of bound water in apple leaves increased towards the end of summer as the heat and drought intensified. Thus, in June, the ratio of bound water to free water was 0.96–1.46, in July – 2.75–5.21, and in August – 6.54–9.95. The Orphey and Margo varieties, identified as highly resistant, consistently had the highest ratios of bound water to free water throughout the summer months.

Elevated temperatures and lack of rainfall can reduce the functional activity of the leaves and the activity of photosynthetic processes, primarily in susceptible plants. This reflects on the content of leaf pigments – chlorophyll and carotenoids. Under drought conditions, the total chlorophyll content in the leaves of two olive tree varieties decreased by 29 and 42%, respectively, compared to the control [8].

In our studies in June, the content of total chlorophylls (*a + b*) in the studied apple varieties depended on varietal characteristics and ranged from 5.81 to 7.04 mg/g dry weight. During the

summer, under the influence of stress factors of the summer vegetation period, the chlorophyll content varied in different varieties and in August was 4.99–6.98 mg/g dry weight. The most stable chlorophyll content was in the Orphey and Margo varieties, indicating the increased adaptability of their leaf tissues (see Fig. 1).

Excessive sunlight can dehydrate and damage the plant leaves. Carotenoids perform a photoprotective function against excessive illumination, and an increase in their proportion in the pigment complex indicates increased stress resistance of the variety. Numerous studies on fruit and other crops have shown that the Chl/Car (chlorophyll/carotenoids) ratio decreased under conditions of drought, increased insolation, and also as the leaf aged [2–5, 9, 10].

In our studies, the content of carotenoids in June was 1.52–2.18 mg/g dry weight depending on the variety. During the summer, the carotenoid content tended to increase, and in August it was 1.58–2.54 mg/g dry weight (see Fig. 2).

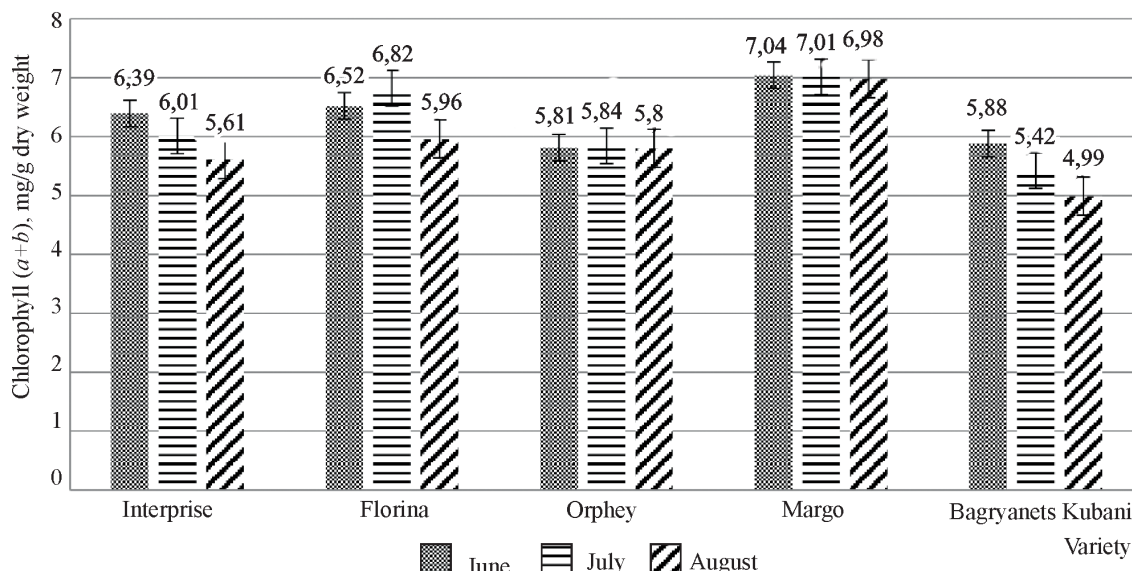
Due to the increased proportion of carotenoids in the leaf pigment composition, the Chl/Car ratio also decreased by the end of summer in all varieties. For example, in June, the maximum value of this indicator was 4.05 in the Enterprise variety, and in August, it was 3.46 in the Enterprise and Florina varieties. It was found that in the Orphey and Margo varieties, the Chl/Car indicators were minimal, and in August they were 2.65 and 2.74, respectively, unlike the other studied varieties, where this ratio varied from 3.15 to 3.46. Based on these data, the Orphey and Margo varieties were identified as highly resistant to heat and drought.

Показатели водного режима листьев яблони летом 2020–2022 гг.

Indicators of the water regime of apple leaves in the summer 2020–2022

Variety	Water content, %			Ratio of bound water to free water		
	June	July	August	June	July	August
Enterprise	62,95 ± 0,38	61,59 ± 0,89	60,55 ± 1,15	0,96 ± 0,05	2,75 ± 0,13	6,87 ± 0,31
Florina	63,55 ± 0,51	62,81 ± 0,13	60,82 ± 0,43	1,02 ± 0,24	3,01 ± 0,02	6,54 ± 0,24
Orphey	64,07 ± 0,49	63,11 ± 0,82	62,62 ± 0,44	1,46 ± 0,03	5,21 ± 0,05	9,95 ± 0,52
Margo	64,23 ± 0,18	63,53 ± 0,30	62,91 ± 1,38	1,28 ± 0,24	4,53 ± 0,02	9,75 ± 0,63
Bagryanets Kubani	64,13 ± 0,60	63,18 ± 0,36	60,86 ± 0,38	0,98 ± 0,15	2,98 ± 0,11	7,46 ± 0,11
LSD <sub>0,5</sub>	1,57	2,43	1,27	1,82	0,43	0,22





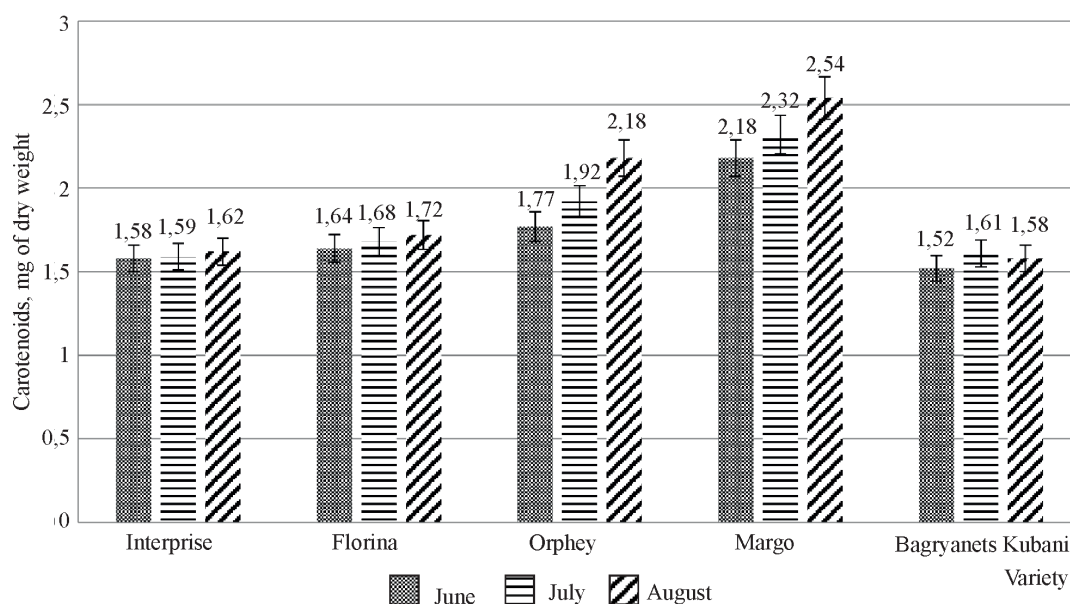
**Рис. 1.** Динамика содержания хлорофилла в листьях яблони в летний период 2020–2022 гг. (средние значения). НСР<sub>05</sub>: июнь – 1,31, июль – 1,50, август – 0,82

**Fig. 1.** Dynamics of chlorophyll content in apple leaves in the summer period 2020–2022 (mean values). LSD<sub>05</sub>: June – 1.31, July – 1.50, August – 0.82

It is known that ascorbic acid (AA) actively participates in water exchange and photosynthesis due to its ability to be reversibly oxidized and reduced, and it is also recognized as an antioxidant [11]. Under its influence, the intensity of transpiration increased in wheat under drought conditions [12]. In grape plants, AA, as a component of the protective antioxidant system,

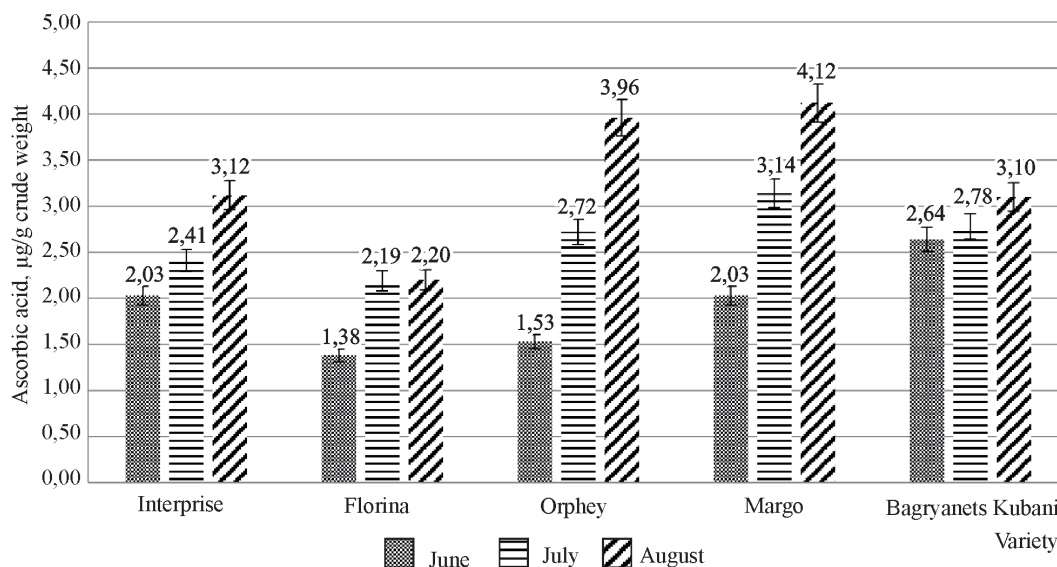
is activated at high positive temperatures and during drought [13].

In our studies, the content of ascorbic acid in June was 1.38–2.64 μg/g fresh weight. During the summer, its content increased, and in August it was 2.20–4.12 μg/g fresh weight. It was found that in the Orphey and Margo varieties, the content of ascorbic acid increased more significant-



**Рис. 2.** Динамика содержания каротиноидов в листьях яблони в летний период 2020–2022 гг. (средние значения). НСР<sub>05</sub>: июнь – 0,32, июль – 0,51, август – 0,28

**Fig. 2.** Dynamics of carotenoids content in apple leaves in summer 2020–2022 (mean values). LSD<sub>05</sub>: June – 0.32, July – 0.51, August – 0.28



**Рис. 3.** Динамика содержания аскорбиновой кислоты в листьях яблони в летний период 2020–2022 гг. (средние значения). НСР<sub>05</sub>: июнь – 0,13, июль – 0,11, август – 0,29

**Fig. 3.** Dynamics of the content of ascorbic acid in apple leaves in the summer period 2020–2022 (mean values). LSD<sub>05</sub>: June – 0.13, July – 0.11, August – 0.29

ly under the stressors of the summer period – by 2.02–2.58 times compared to other studied varieties, in which this increase was 1.17–1.59 times (see Fig. 3).

A similar effect was observed in tea leaves in July and August, when the AA content increased as the plants adapted to extremely high temperatures and drought<sup>5</sup>.

## CONCLUSIONS

As a result of the assessment of five apple varieties of various ecological and geographical origins for heat and drought resistance, the domestically bred Orphey and Margo varieties were identified as highly resistant based on the identified physiological and biochemical characteristics of the leaf:

- the least decrease in the water content of leaf tissues (by 1.32–1.45%) compared to other studied varieties, which had a decrease of 2.40–3.27%;
- the highest indicators of the ratio of bound water to free water throughout the summer

months, which in August were 9.95 and 9.97, respectively, while in other varieties they were 6.54–7.46;

- stable content of total chlorophylls during the summer, indicating the increased adaptability of leaf tissues;

- a low ratio of total chlorophylls to carotenoids, which was 2.65 and 2.74 in August, respectively, compared to other studied varieties, where it varied from 3.15 to 3.46;

- an increase in ascorbic acid content to a greater degree (by 2.02–2.58 times) compared to other studied varieties, which had an increase of 1.17–1.59 times.

The Orphey and Margo varieties proved to be more adaptive for cultivation in the Krasnodar Territory conditions. They can be used in breeding to produce varieties that are more resistant to heat and drought.

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#### ИНФОРМАЦИЯ ОБ АВТОРАХ

✉ **Киселева Г.К.**, кандидат биологических наук, доцент, старший научный сотрудник; **адрес для переписки:** Россия, 350901, Краснодарский край, Краснодар, ул. им. 40-летия Победы, 39; e-mail: galina-kiseleva-1960@mail.ru

**Ульяновская Е.В.**, доктор сельскохозяйственных наук, заведующая лабораторией

**Схаляхо Т.В.**, младший научный сотрудник

**Каравеева А.В.**, младший научный сотрудник

#### AUTHOR INFORMATION

✉ **Galina K. Kiseleva**, Candidate of Science in Biology, Associate Professor, Senior Researcher; **address:** 39, 40-letiya Pobedy St., Krasnodar, Krasnodar Territory, 350901, Russia; e-mail: galina-kiseleva-1960@mail.ru

**Elena V. Ulyanovskaya**, Doctor of Science in Agriculture, Laboratory Head

**Tatyana V. Skhalyaho**, Junior Researcher

**Alla V. Karavaeva**, Junior Researcher

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## ПЕРСПЕКТИВНЫЕ ФУНГИЦИДЫ ДЛЯ ЗАЩИТЫ ЯРОВОГО РАПСА ОТ СЕМЕННОЙ ИНФЕКЦИИ И КОРНЕВЫХ ГНИЛЕЙ НА СЕВЕРО-ЗАПАДЕ РОССИЙСКОЙ ФЕДЕРАЦИИ

✉ Шпанев А.М., Гасич Е.Л.

*Всероссийский научно-исследовательский институт защиты растений*

Санкт-Петербург, Россия

✉ e-mail: ashpanev@mail.ru

Представлены результаты изучения эффективности новых фунгицидов, предназначенных для защиты ярового рапса от семенной инфекции и корневых гнилей путем обработки посевного материала, в природно-климатических условиях Северо-Запада Российской Федерации. В последние годы произошло существенное увеличение посевных площадей ярового рапса в регионе. Одновременно с этим наметилась тенденция к ухудшению фитосанитарного состояния посевов данной культуры. В условиях накопления инфекции фунгицидная обработка семян будет все более востребованной. По результатам исследований определено, что новые препараты Тирада, СК (норма расхода 2,0 и 3,0 л/т), Витарос, ВСК (3,0; 4,0 и 6,0 л/т) и Синклер, СК (1,3 и 1,6 л/т) являются более эффективными для снижения семенной инфекции (*Alternaria* spp. – 86,6–94,9; 93,2–96,6; 91,7–100% соответственно; плесневые грибы – 86,7–93,4; 90,9–96,6; 73,5–78,6%) и развития корневых гнилей (фаза формирования четырех настоящих листьев – 37,7–56,7; 27,2–52,3; 31,7–72,7%; фаза стеблевания – 13,4–41,2; 17,9–29,0; 17,9–50,4%), чем применяемые в регионе фунгициды Скарлет, МЭ и Редут, КС. Обработка семян рапса фунгицидами способствовала росту урожайности культуры, которая в зависимости от препарата и нормы его расхода оказалась на 3,0–50,0% выше, чем в контроле. Полученные по результатам опытов показатели биологической и хозяйственной эффективности свидетельствуют о значительных перспективах использования новых фунгицидов в защите ярового рапса от семенной и почвенной инфекции путем обработки посевного материала в условиях северо-западной части России.

**Ключевые слова:** яровой рапс, семенная инфекция, корневые гнили, обработка семян, фунгициды, биологическая эффективность, Северо-Запад РФ

## PROMISING FUNGICIDES FOR PROTECTING SPRING RAPE AGAINST SEED INFECTION AND ROOT ROTS IN THE NORTH-WEST OF THE RUSSIAN FEDERATION

✉ Shpanev A.M., Gasich E.L.

*All-Russian Institute of Plant Protection*

Saint Petersburg, Russia

✉ e-mail: ashpanev@mail.ru

The results of studying the effectiveness of new fungicides designed to protect spring rape from seed infection and root rot by treatment of the seed material in natural-climatic conditions of the North-West of the Russian Federation are presented. In recent years, there has been a significant increase in the area sown to spring rape in the region. At the same time, a trend towards deterioration of the crop's phytosanitary condition has been observed. With the accumulation of the infection, fungicide seed treatment will be increasingly required. Based on the results of the research, it has been determined that the new preparations Tirada, SC (application rate 2.0 and 3.0 l/t), Vitaros, WSC

(3.0; 4.0 and 6.0 l/t) and Sinkler, SC (1.3 and 1.6 l/t) are more effective in reducing the seed infection (*Alternaria* spp. – 86.6–94.9; 93.2–96.6; 91.7–100%, respectively; mold fungi – 86.7–93.4; 90.9–96.6; 73.5–78.6%) and root rot development (four true leaves formation phase – 37.7–56.7; 27.2–52.3; 31.7–72.7%; stemming phase – 13.4–41.2; 17.9–29.0; 17.9–50.4%) than Scarlet, ME and Redut, SC fungicides used in the region. Treatment of the rape seeds with fungicides contributed to the growth of the crop yield, which depending on the preparation and its consumption rate was 3.0–50.0% higher than in the control. The indices of the biological and economic efficiency obtained according to the results of the experiments testify to the significant prospects of using new fungicides in the protection of spring rape from seed and soil infection by treatment of the sowing material in the conditions of the north-western part of Russia.

**Keywords:** spring rapeseed, seed infection, root rots, seed treatment, fungicides, biological effectiveness, North-West of the Russian Federation

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**Конфликт интересов**

Авторы заявляют об отсутствии конфликта интересов.

**Conflict of interest**

The authors declare no conflict of interest.

## INTRODUCTION

Today rapeseed is the most dynamically spreading crop on the territory of Russia. The main rapeseed acreage is located in the Siberian Federal District, the Volga, Central, Volga-Vyatka and Ural districts are also among the top five [1]. The North-Western region by its natural and climatic conditions is favorable for rapeseed cultivation, but until recently the sown areas of this crop here were extremely small and were limited exclusively to the Kaliningrad region, where the winter form prevails. Since 2016, there has been a noticeable increase in the sown areas of spring rape mainly due to the Pskov, Novgorod and Leningrad regions [2]. Against the background of significant expansion of the cultivated areas, there has been a significant deterioration of phytosanitary condition of crops, which manifested in the growth of cases of mass reproduction of pests and epiphytotic development of diseases [3]. Taking into account the emerging tense phytosanitary situation, the implementation of protective measures becomes especially demanded, as well as the studies to assess the effectiveness of new preparations for the protection of rape from pests, diseases and weeds.

The integrated system of spring rape protection from pests includes pre-sowing seed treatment with insecticidal, insecto-fungicidal or only fungicidal preparations [4, 5]. Taking into account the peculiarities of phytosanitary situation treatment of seed material with fungicides is currently not mandatory. However, in the conditions of the emerging trends towards a significant expansion of the sown areas and the emergence of more and more farms practicing long-term cultivation of spring and even winter rape, fungicide treatment of seeds will be more and more in demand. Its main purpose is associated with the provision of quality protection of seedlings and young plants of spring rape from pathogens present on the seeds and in the soil [6]. This technique contributes to the increase of field germination, density of productive plants and, as a consequence, the formation of higher yields, which is confirmed by numerous data from different zones of cultivation of the crop [7-9].

The purpose of the study is to evaluate the effectiveness of new fungicides designed to protect spring rape from seed infection and root rots by treatment of seed material, in natural-climat-

ic conditions of the North-West of the Russian Federation.

## MATERIAL AND METHODS

The research was conducted in 2019-2021 both in laboratory and field conditions. The object of study were new fungicidal preparations for rape seed treatment, created by the specialists of the company "August". These preparations have different active substances in their composition: Tirada, SC (400 g/l thiram + 30 g/l diphenyl-conazole; consumption rate 2.0 and 3.0 l/t), Vitaros, WSC (198 g/l carboxin + 198 g/l thiram; rate 3.0, 4.0 and 6.0 l/t), Sinkler, SC (75 g/l fludioxonil; rate 1.3 and 1.6 l/t). Scarlet, ME (100 g/l imazalil + 60 g/l tebuconazole) and Redut, SC (60 g/l tebuconazole) at rates of 0.4 and 0.5 L/t, respectively, were used as reference formulations currently used on spring rape. Seed treatment was carried out at a working fluid consumption of 10.0 l/t. The control in the experiments was a variant in which no fungicide treatment of seeds was provided.

The effect of rape seed treatment with fungicides on the presence of seed infection was determined in laboratory conditions. To determine the infection of seeds with pathogens, the nutrient media technique was used<sup>1</sup>. Each variant consisted of 100 seeds placed in Petri dishes with 10 pieces in tenfold repetition.

Field studies were conducted at the biopolygon of the Menkovskaja branch of the Agrophysical Research Institute, located in the Gatchina district of the Leningrad region. The soil of the experimental fields of the test site was soddy-weak podzolic sandy loam, arable layer thickness - 23 cm,  $pH_{KCl} = 4.6$ , humus content (according to Tyurin) - 1.9%, mobile compounds of phosphorus and potassium (according to Kirсанов) - 257 and 92 mg/kg, respectively.

The methodological basis was the recommendations for assessing the effectiveness of fungicides in seed treatment<sup>2</sup>. The size of the plots in the experiments was 2 m<sup>2</sup>, placement was ran-

domized, repetition was fourfold. Soil preparation consisted of autumn plowing, cultivation, pre- and post-sowing rolling. Sowing was carried out manually at the rate of 8 kg of seed/ha. Assumed density of 120 plants/m<sup>2</sup>. Crop care measures included background treatments with insecticides and herbicides. Monitoring of root rot development was conducted twice: in the phase of formation of four true leaves and the phase of stemming. In both cases, at least 50 plants from each plot were subject to inspection. The intensity of root rot lesions on each plant was determined. To assess the level of lesion, a point scale was used, according to which 1 point corresponded to a weak lesion (brown stripes were noticeable on the root), 2 points - to a medium lesion (the root began to form a tangle), 3 points - to a strong lesion (the tangle covered more than half of the root), and 4 points - to the death of the plant.

Biological efficiency of rape seed treatment with fungicides was calculated using the Abbott formula (see footnote 2).

The yield was measured at the phase of full ripeness by cutting plants from 1 m<sup>2</sup> of each plot and threshing in laboratory conditions. The density of productive and unproductive plants, total seed weight, seed weight per plant, and 1000 seed weight were determined.

Statistical processing of the data was carried out with the help of Statistica 6.0 program and consisted in the application of dispersion analysis to detect reliable differences in the manifestation of diseases and the emerging yield of spring rape between the variants of the experiments.

## RESULTS AND DISCUSSION

Phytoexamination of the seeds revealed the presence of fungi of the genus *Alternaria*, as well as *Penicillium* spp., *Aspergillus* spp., *Cladosporium* spp. and *Mucor* spp. causing mold. Indicators of seed infestation on the control not treated with fungicides indicate that the evaluation of drug efficacy was carried out under the

<sup>1</sup>GOST 12044-93. Interstate standard. Seeds of agricultural crops. Methods of determination of disease infestation. Moscow, 1993, 58 p.

<sup>2</sup>Methodical guidelines for registration tests of fungicides in agriculture. St. Petersburg: Publishing house of the All-Russian Research Institute of Plant Protection, 2009, 378 p.

conditions of weak, moderate and strong infectious load. Thus, the infection of seeds with *Alternaria* spp. fungi varied by years from 6.0 to 59.0%, mold fungi - from 12.0 to 98.0%.

According to the results of phytoexpertise, it was determined that the preparations Scarlet, ME and Redut, SC used as standards provided reliable protection only against *Alternaria* fungi: reduction of seed infestation amounted to 91.5-100.0 and 85.7-100.0%, respectively. The protective effect against mold fungi was much weaker: 50.0-90.0 and 41.8-50.0% (see Table 1).

Compared to the reference preparations, the new fungicides demonstrated similar efficacy against *Alternaria* spp. fungi and higher efficacy against mold fungi. Reduction of seed infection by *Alternaria* fungi in the variant with treatment with Tirada, SC according to averaged two-year test data depending on the rate of consumption was 86.6-94.9%, by mold fungi - 86.7-93.4%, in treatment with Vitaros, WSC - 93.2- 96.6 and 90.9-96.6%, Sinkler, SC - 91.7-100.0 and 73.5-78.6%. It should be noted that the effectiveness of the dressing agents increased with the reduction of the infection load.

The process of seed infection development in different variants of the experiment is presented in Fig. 1.

Root rot is manifested on seedlings and young plants of spring rape in the form of darkening

and thinning of the stem in the area of the root neck (see Fig. 2). In case of severe lesions, roots die off, plants lag behind in development, have chlorotic or anthocyanin coloration of leaves and may die [10, 11]. The disease is caused both by one species and a whole complex of species of micromycetes. In the Leningrad Region, plants are more often affected by *Rhizoctonia solani* J.G. Kühn. as well as by *Fusarium* spp. (*F. avenaceum*, *F. equiseti*, *F. oxysporum*, etc.) and *Pythium* spp. Root rot is sometimes caused by the infection of roots and root neck by mycelium developed from sclerotia of *Sclerotinia sclerotiorum* (Libert) de Bary in the soil. In our experiments, fungi of the genus *Fusarium* were most often isolated in pure culture.

The efficiency of fungicide treatment of rape seed was evaluated in the absence of *Fusarium* fungi on seeds, against the background of weak and moderate development of root rot, caused exclusively by soil infection. In the control variant in the phase of formation of four true leaves, the proportion of plants affected by root rot varied by years within the limits of 5.4-11.5%, in the shooting stage- 9.9-13.4%.

The efficiency of the reference preparations Scarlet, ME and Redut, SC in rape protection against root rots was low and did not exceed 31.0% in both phases. Higher indicators were observed in new preparations. Reduction of root

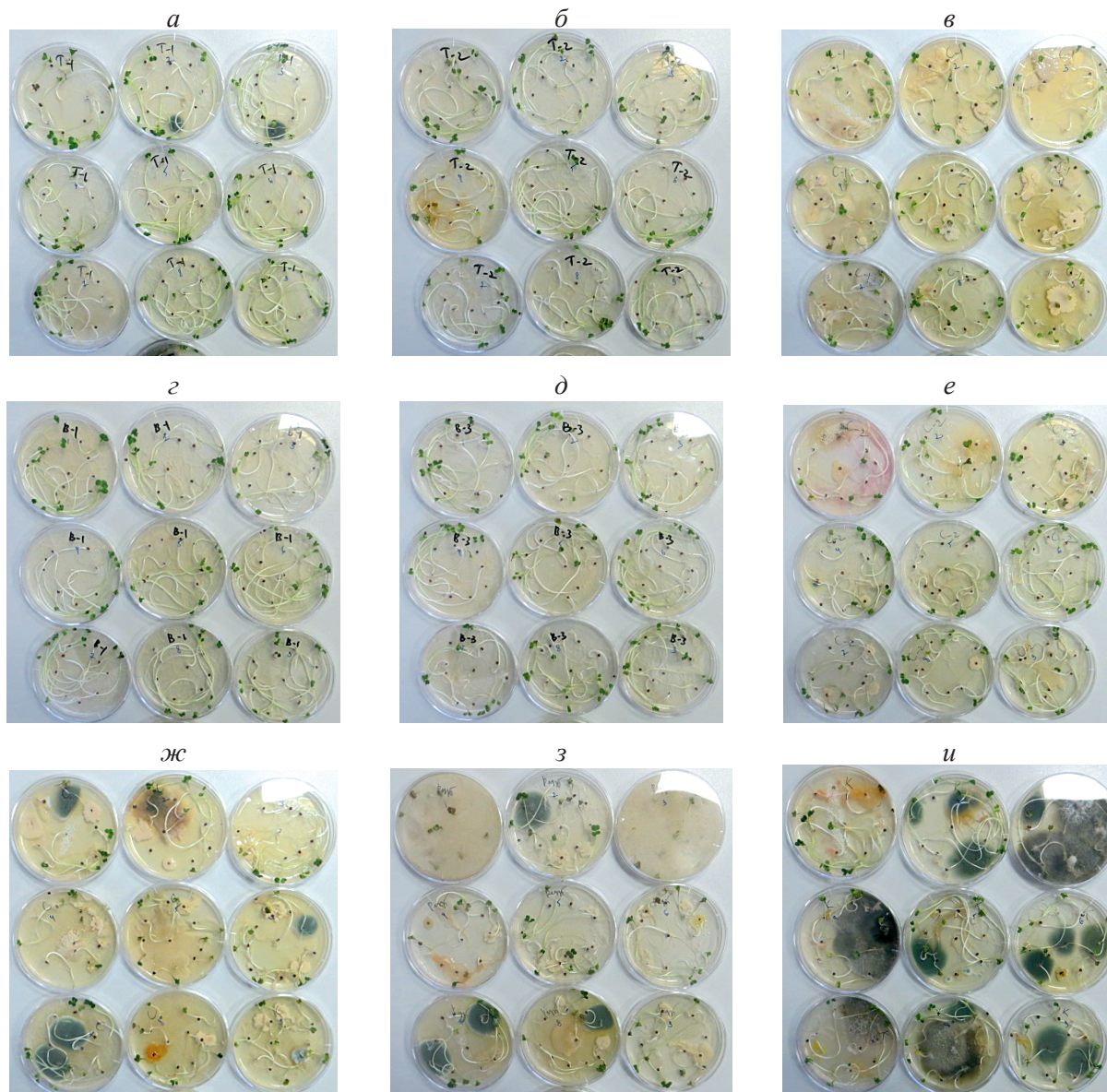
**Табл. 1.** Биологическая эффективность фунгицидов против семенной инфекции при обработке семян ярового рапса

**Table 1.** Biological efficiency of fungicides against seed infection in the treatment of spring rape seeds

Experiment option	Consumption rate, l/t	Reduction of infestation relative to the control, %					
		2019		2020		2021	
		<i>Alternaria</i> spp.	Mold fungi	<i>Alternaria</i> spp.	Mold fungi	<i>Alternaria</i> spp.	Mold fungi
Tirada, SC	2,0	89,8	90,0	83,3	83,3	—	—
	3,0	89,8	95,0	100,0	91,7	—	—
Vitaros, WSC	3,0	86,4	90,0	100,0	91,7	—	—
	4,0	93,2	95,0	100,0	91,7	—	—
	6,0	93,2	100,0	100,0	100,0	—	—
Sinkler, SC	1,3	—	—	83,3	100,0	100,0	46,9
	1,6	—	—	100,0	100,0	100,0	57,1
Scarlet, ME	0,4	91,5	90,0	100,0	50,0	—	—
Redut, SC	0,5	—	—	100,0	50,0	85,7	41,8
Without treatment (control)*	—	59,0	20,0	6,0	12,0	7,0	98,0

\* Actual values of seed infestation, %.





**Рис. 1.** Проявление инфекции на семенах ярового рапса в вариантах без обработки и с обработкой фунгицидами:

*а, б* – Тирада, СК (норма расхода 2,0 и 3,0 л/т); *в, е* – Синклер, СК (1,3 и 1,6 л/т); *г, д* – Витарос, ВСК (3,0 и 6,0 л/т); *ж* – Скарлет, МЭ (0,4 л/т); *з* – Редут, КС (0,5 л/т); *и* – контроль

**Fig. 1.** The manifestation of infection on spring rape seeds in the variants without treatment and with seed treatment with fungicides:

*а, б* – Tirada, SK (2.0 and 3.0 l/t); *в, е* – Sinclair, SK (1.3 and 1.6 l/t); *г, д* – Vitaros, VSK (3.0 and 6.0 l/t); *ж* – Scarlet, ME (0.4 l/t); *з* – Redout, KS (0.5 l/t); *и* – control

rot infestation in the phase of formation of four true leaves in the variant with seed treatment with Tirada, SC according to averaged two-year data depending on the rate of consumption was 37.7-56.7%, in the shooting phase - 13.4-41.2%, in case of treatment with Vitaros, WSC - 27.2-52.3 and 17.9-29.0%, Sinkler, SC - 31.7-72.7 and 17.9-50.4%. It should be noted that in all cases the best result was observed at higher

rates of consumption of preparations. The effectiveness of dressing agents decreased with the growth and development of plants, which can be seen when comparing the data of root rot development in both phases (see Table 2).

Seed treatment with fungicides also contributed to the growth of the rapeseed yield by 3.0-50.0% relative to the control. The increase in yield (by 14.0% according to the averaged



**Рис. 2.** Пораженные (*a*) и не пораженные (*б*) корневой гнилью растения ярового рапса  
**Fig. 2.** Root rot affected (*a*) and not affected (*б*) spring rape plants

**Табл. 2.** Биологическая эффективность фунгицидов против корневых гнилей при обработке семян ярового рапса

**Table 2.** Biological efficiency of fungicides against root rot in the treatment of spring rape seeds

Experiment option	Consumption rate, l/t	Reduction of root rot development relative to the control, %					
		2019		2020		2021	
		Formation of four true leaves	Shooting	Formation of four true leaves	Shooting	Formation of four true leaves	Shooting
Tirada, SC	2,0	36,2	12,6	39,2	14,2	–	–
	3,0	45,7	41,4	67,6	41,0	–	–
Vitaros, WSC	3,0	29,6	21,2	24,7	14,5	–	–
	4,0	41,7	23,2	37,0	31,8	–	–
	6,0	53,9	25,3	50,6	32,7	–	–
Sinkler, SC	1,3	–	–	39,2	14,2	24,1	21,5
	1,6	–	–	67,6	41,0	77,8	59,8
Scarlet, ME	0,4	25,2	31,3	6,8	15,7	–	–
Redut, SC	0,5	–	–	29,6	5,6	18,5	16,8
Without treatment (control)*	–	11,5	9,9	7,4	13,4	5,4	10,7

\*Factual values of root rot development, %.

data of the experiments) was mainly due to such an element of the yield structure as seed weight per plant, to a lesser extent - due to the density of productive plants (by 8.0%) and 1000 seeds weight (by 7.0%).

The highest safety of the rapeseed yield was observed in 2021 in the variants with the use of Sinkler, SC in conditions of heavy infestation of seed with mold fungi, which led to a decrease in the density of total and productive stalks. In this case, according to the results obtained, the preparation-ethalon Redut, SC was inferior in economic efficiency to the new fungicide Sinkler, SC depending on the rate of consumption of the latter in 1.2-1.4 times. In 2020, reliable differences in yields were observed only between the variants with application of fungicides Sinkler, SC at a rate of 1.6 l/ha and Redut, SC - 64.1 and 56.3 g/m<sup>2</sup>, respectively (see Table 3). During two years of experiments, higher indicators of economic efficiency, in comparison with the reference preparation Scarlet, ME, were observed when the seeds were treated with the new fungicide Vitaros, WSC at concentrations of 4.0 and 6.0 l/t. The smallest economic effect was obtained when the seeds were treated with Tirada, SC. In this case, there were no reliable differenc-

es in yield of spring rape seeds not only with the standard, but also with the control.

## CONCLUSIONS

1. In the process of research in natural-climatic conditions of North-West Russia, high efficiency of new fungicides application for protection of spring rape seeds from seed infection and average efficiency in relation to root rots of fusarium etiology was confirmed. At the same time, as a result of the use of new preparations, a higher level of reduction of disease development was observed than in variants with already authorized fungicides Scarlet, ME and Redut, SC. The value of the preserved yield when treated with Tirada, SC (consumption of 2.0 and 3.0 l/t) according to the averaged data for two years was 5.1 and 13.0 g/m<sup>2</sup> (5.0 and 10.0%), when treated with Vitaros, WSC (consumption of 3.0; 4.0 and 6.0 l/t) - 8.3; 20.5 and 22.5 g/m<sup>2</sup> (9.0; 25.0 and 27.0%), Sinkler, SC (1.3 and 1.6 l/t) - 7.6 and 14.0 g/m<sup>2</sup> (24.0 and 42.0%), respectively.

2. The obtained indicators of biological and economic efficiency indicate great prospects for the use of new fungicides in the protection of spring rape from seed and soil infection by treatment of seed material in the North-West of the

**Табл. 3.** Влияние обработки семенного материала фунгицидами на урожайность ярового рапса  
**Table 3.** Effect of seed treatment with fungicides on spring rape yields

Experiment option	Consumption rate, l/t	2019		2020		2021	
		g/m <sup>2</sup>	% to the control	g/m <sup>2</sup>	% to the control	g/m <sup>2</sup>	% to the control
Tirada, SC	2,0	159,4	105,0	85,8	104,0	–	–
	3,0	172,8	113,0	88,2	107,0	–	–
Scarlet, ME	0,4	180,6	118,0	84,9	103,0	–	–
Without treatment (control)	–	152,5	100,0	82,5	100,0	–	–
LSD <sub>05</sub>		24,5		17,1			
Vitaros, WSC	3,0	134,3	109,0	70,9	109,0	–	–
	4,0	142,6	115,0	87,0	134,0	–	–
	6,0	144,2	117,0	89,4	137,0	–	–
Scarlet, ME	0,4	136,8	111,0	72,2	111,0	–	–
Without treatment (control)	–	123,6	100,0	65,1	100,0	–	–
LSD <sub>05</sub>		19,3		15,5			
Sinkler, SC	1,3	–	–	55,6	115,0	31,9	132,0
	1,6	–	–	64,1	133,0	36,2	150,0
Redut, SC	0,5	–	–	56,3	117,0	25,8	107,0
Without treatment (control)	–	–	–	48,3	100,0	24,1	100,0
LSD <sub>05</sub>		–		7,8		5,9	

Russian Federation. Registration of the fungicides Tirada, SC, Vitaros, WSC and Sinkler, SC as preparations authorized for rapeseed treatment will open up opportunities for their widespread use, especially in demand in the conditions of the predicted deterioration of phytopathological situation.

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## ИНФОРМАЦИЯ ОБ АВТОРАХ

✉ Шпанев А.М., доктор биологических наук, ведущий научный сотрудник; **адрес для переписки:** Россия, 196608, г. Санкт-Петербург, Пушкин, ш. Подбельского, 3; e-mail: ashpanev@mail.ru

Гасич Е.Л., кандидат биологических наук, старший научный сотрудник

## AUTHOR INFORMATION

✉ Alexander M. Shpanev, Doctor of Science in Biology, Lead Researcher; **address:** 3, Podbel'skogo St., Pushkin, St. Petersburg, 196608, Russia; e-mail: ashpanev@mail.ru

Elena L. Gasich, Candidate of Science in Biology, Senior Researcher

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## КЛОПЫ *COREUS MARGINATUS ORIENTALIS* KIR. И *MOLIPTERYX FULIGINOSA* UHL. (HETEROPTERA, COREIDAE) – ВРЕДИТЕЛИ КУЛЬТУРНЫХ РОЗОВЫХ (ROSACEAE) В ПРИМОРСКОМ КРАЕ

✉ Маркова Т.О., Маслов М.В.

Федеральный научный центр биоразнообразия наземной биоты Восточной Азии

Дальневосточного отделения Российской академии наук

Владивосток, Россия

✉ e-mail: martania@mail.ru

Приведены сведения о биологии и экологии клопов *Coreus marginatus orientalis* и *Molipteryx fuliginosa* (Heteroptera, Coreidae), которые за последние 10 лет стали серьезными вредителями в антропо- и агроценозах юга Дальнего Востока России. Цель исследования – обобщить данные наблюдений за этими видами как вредителями культурных Розовых в Приморском крае. Исследования проводили с мая по октябрь 2007–2022 гг.: осуществляли сбор материала полужесткокрылых, наблюдения в естественных местообитаниях и стационарных условиях. *M. fuliginosa* отмечен на окультуренных территориях Уссурийского городского округа на *Rosa acicularis*, *R. rugosa*, *R. davurica*, *Rubus komarovii*, *R. crataegifolius*, используемых в озеленении. Зафиксированы случаи питания клопов, высасывания сока из вегетативных частей растений и соплодий. *C. m. orientalis* обнаружен на приусадебных участках в Чугуевском, Октябрьском, Ханкайском районах и Уссурийском городском округе. Собран на кустах *Rubus idaeus*, *R. caesius*, которые являются общими объектами питания для *C. m. orientalis* и *M. fuliginosa*. На культивируемых растениях происходят скопление, копуляция и яйцекладка этих насекомых. В результате заселения растений *M. fuliginosa* нарушается нормальное развитие листовых пластинок, наблюдается увядание апикальной части побегов. При питании *C. m. orientalis* на соплодиях малины происходят повреждение цветоложа и усыхание костянок.

**Ключевые слова:** Heteroptera, Coreidae, Дальний Восток России, Приморский край, *Coreus marginatus orientalis*, *Molipteryx fuliginosa*, Rosaceae

## LEAF-FOOTED BUGS *COREUS MARGINATUS ORIENTALIS* KIR. AND *MOLIPTERYX FULIGINOSA* UHL. (HETEROPTERA, COREIDAE) – PESTS OF CULTIVATED ROSES (ROSACEAE) IN THE PRIMORSKY TERRITORY

✉ Markova T.O., Maslov M.V.

Federal Scientific Center of the East Asia Terrestrial Biodiversity, Far Eastern Branch  
of the Russian Academy of Sciences

Vladivostok, Russia

✉ e-mail: martania@mail.ru

The data on biology and ecology of bed bugs *Coreus marginatus orientalis* and *Molipteryx fuliginosa* (Heteroptera, Coreidae), which have become serious pests in anthropo- and agroecosystems of the south of the Russian Far East for the last 10 years, are presented. The purpose of the study is to summarize the observation data on these species as pests of cultivated Rose species in the Primorsky Territory. The studies were carried out from May to October 2007–2022: material collection of hemipterans, observations in natural habitats and stationary conditions were conducted. *M. fuliginosa* was observed in the cultivated areas of the Ussuriysky urban district on *Rosa acicularis*, *R. rugosa*, *R. davurica*, *Rubus komarovii*, *R. crataegifolius* used in landscaping. Cases of bed bugs feeding, sucking sap from vegetative parts of the plants and seedballs were recorded. *C. m. orientalis* was found on homestead plots in the Chuguevsky, Oktyabrsky, Khankaisky districts and the Ussuriysky urban district. It was collected on the bushes of *Rubus idaeus*, *R. caesius*, which are common feeding sites for *C. m. orientalis* and *M. fuliginosa*. Aggregation, copulation and oviposition of these insects occur on the cultivated plants. As a result of the infestation of plants by *M. fuliginosa*, normal development of leaf plates is disturbed, wilting of the apical part of shoots is observed. When *C. m. orientalis* feeds on raspberry seedballs, damage to the flower disc and desiccation of the drupes occur.

**Keywords:** Heteroptera, Coreidae, Russian Far East, Primorsky Territory, *Coreus marginatus orientalis*, *Molipteryx fuliginosa*, Rosaceae

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## INTRODUCTION

A serious ecological problem in the territory of the Far Eastern Federal District and Russia as a whole is the introduction of alien species into the composition of natural and anthropogenically-transformed biocenoses [1–5]. These invasive species can form stable populations, create competition with native fauna species, and acquire the status of pests. In recent years, we have been observing the biology and ecology of the bugs *Molipteryx fuliginosa* (Uhler, 1860) and *Coreus marginatus orientalis* (Heteroptera, Coreidae) (Kiritshenko, 1916) in the conditions of the Primorsky Territory [6, 7]. The native fauna representative *C. m. orientalis* has several Russian names (kraevik, or rombovik okaimlenny (leaf-footed bug); sorrel, or rhubarb bug) and is one of the background species in the south of the Russian Far East. To date, *C. m. orientalis*

has not been included in the list of agricultural pests in the Far East<sup>1,2</sup>.

*M. fuliginosa* was recorded in the Russian Far East more than three decades ago<sup>3</sup>. In China, it is included in the list of economically significant insects<sup>4</sup>, but is not considered a primary pest, as its host plants are weed species. This list also includes *Camellia oleifera* Abel (Theaceae) [8], *Bambusa* sp. (Poaceae, Bambusoideae)<sup>5</sup>, and *Oryza* sp. (Poaceae) [9]. In recent years, *M. fuliginosa* has been expanding its range and becoming harmful to garden plants. A mass increase in the bug's population in several districts of the Primorsky Territory was noted in 2012 and 2015. The insect was observed feeding on raspberries (*Rubus* sp.), which prompted close attention and inclusion in the list of invasive insects [10, 11]. In recent years, adults and larvae of *C. m. orientalis* and *M. fuliginosa* are increas-

<sup>1</sup>Kanyukova E.V. Order Heteroptera - Hemipterans or bedbugs // Insects - pests of agriculture of the Far East. Vladivostok: Dalnauka, 1995, pp. 51–55.

<sup>2</sup>Mishchenko A.I. Insects - pests of agricultural plants of the Far East. Khabarovsk, 1957, 205 p.

<sup>3</sup>Kerzhner I.M., Kanyukova E.V. First record of *Molipteryx fuliginosa* Uhler from Russia (Heteroptera: Coreidae) // Zoosystematica Rossica. 1998, vol. 7, N 1, p. 84.

<sup>4</sup>Zhang Sh. Economic Insect Fauna of China // Hemiptera. 1985, vol. 1, Fasc. 31, pp. 1–242, I–LIX.

<sup>5</sup>Wang H.J., Li P., Gao Y.D., Yuan M. A list of insect pests of bamboos in Baishuijiang Natural Reserve // Journal of Gansu Forestry Science and Technology. 2002, vol. 27, N 4, pp. 12–16.

ingly found together in agroecosystems of the southern Primorsky Territory and are trending towards becoming pests.

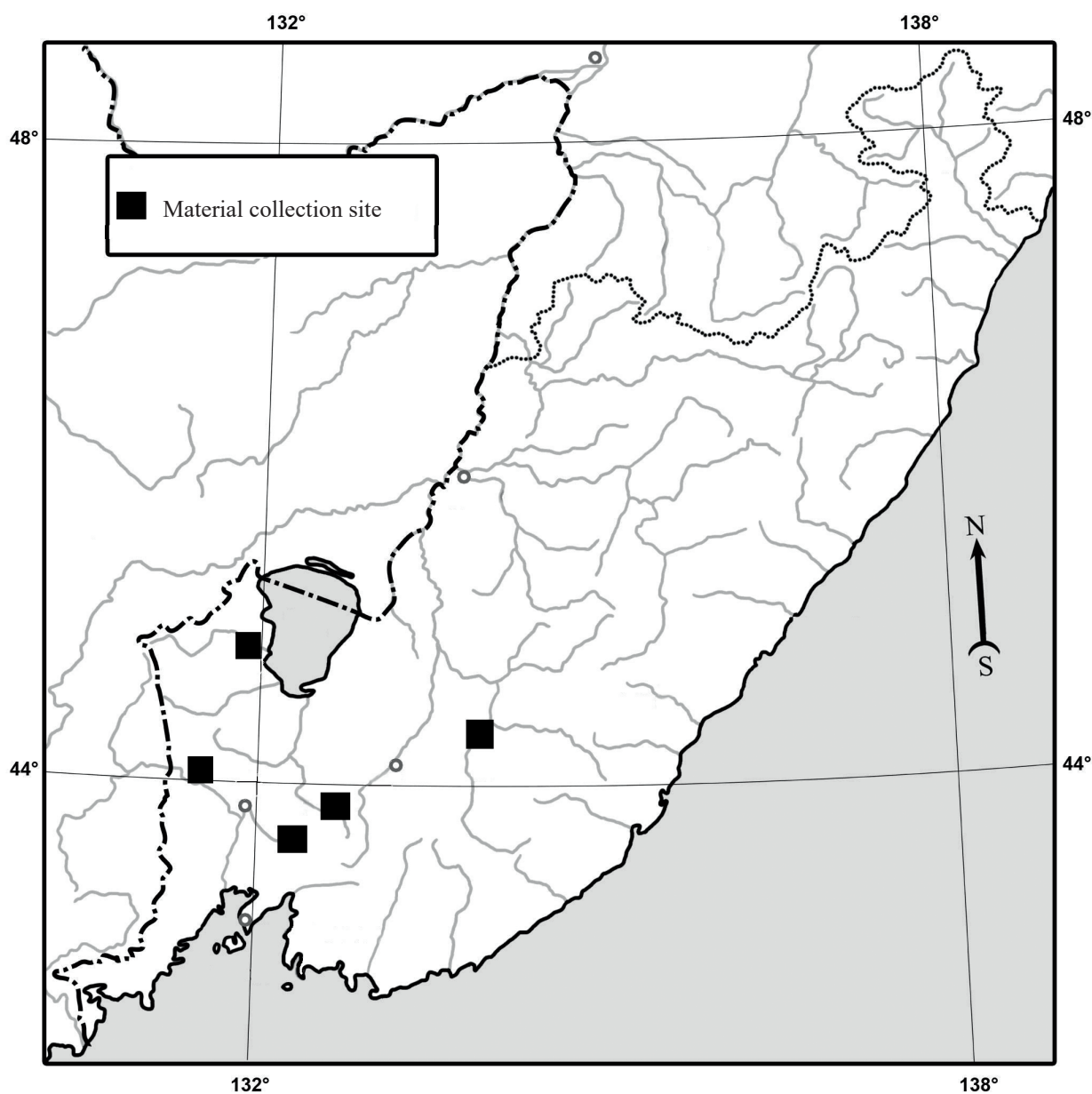
The purpose of the study is to summarize observational data and present new information on the bugs *C. m. orientalis* and *M. fuliginosa* as pests of cultivated Rosaceae in the Primorsky Territory.

## MATERIAL AND METHODS

The research was conducted from May to October in 2007–2022 in the Primorsky Territory

(see Fig. 1). It involved collecting specimens, observing insects in their natural habitats and under controlled conditions (some bugs were kept in cages). The methods for collecting and keeping insects were previously specified [6, 7].

Comparative observations of the insects were made in cages and natural cenoses from the emergence of overwintered adults in spring and the beginning of egg-laying until the hatching of larvae, their maturation, molts of all five ages, up to the development of new generation adults. All processes were accompanied by photo shooting.



**Рис. 1.** Карта района исследования с указанием мест сбора материала  
**Fig. 1.** Map of the study area with the locations of material collection



In natural conditions, the insects' feeding behavior was observed, and symptoms of fruit damage, wilting, and drying of plant parts above the feeding site were identified.

The materials are partially stored in T.O. Markova's personal collection and the collection of the Zoological Museum (Far Eastern Federal District, Vladivostok).

## RESULTS AND DISCUSSION

*M. fuliginosa* (see Fig. 2) in the Primorsky Territory is noted in the following cultivated habitats and agrocenoses of the Ussuriysk urban district:

1. Household plots in the villages of Kamenushka, Kaymanovka, Gornotaizhnoe. Collected on bushes of common raspberry (*Rubus idaeus* L.), remontant raspberry (*R. sp.*), and dewberry (*R. caesius* L.), 20.V–27.IX.2015, 30.V–30.IX.2016–2022, total: 700 specimens (T.O. Markova, M.V. Maslov).

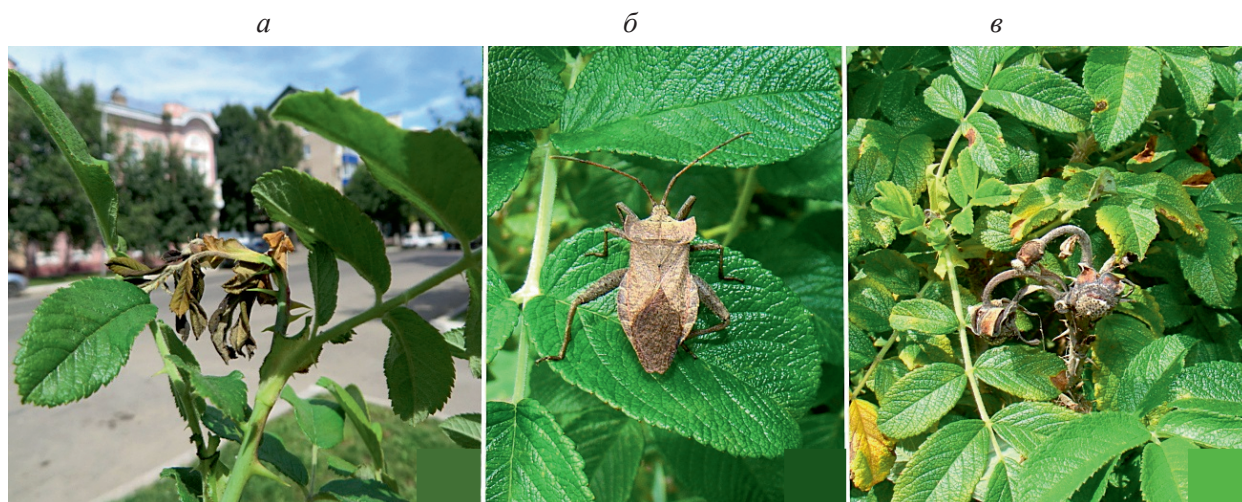
2. Greened areas in the villages of Kamenushka, Kaymanovka. Collected on the bushes of rugosa rose (*Rosa rugosa* Thunb.), hawthorn raspberry (*Rubus crataegifolius* Bunge), 03.IX.2015, VI–IX.2016, VII–VIII.2019, total: 25 specimens (T.O. Markova, M.V. Maslov, L.N. Fedina).

3. Fruit nurseries in Zarechnoe village (in the "Rodina" horticultural association's fruit nursery, S.A. Makarevich grows seedlings of fruit trees, berry bushes, and grapes, all cultures grow separately). Collected in the raspberry seedling propagation field, on bushes, 23.VI.2020, total: 3 specimens (S.A. Makarevich).

4. Greened areas in Ussuriysk (central part of the city – Oktyabrskaya, Plekhanova, Sovetskaya streets, outskirts – Pologaya street). In recent years, various species of Rosaceae have been used for greening – Daurian rose, prickly rose (*Rosa acicularis* Lindl.), Komarov's raspberry (*Rubus komarovii* Nakai), etc. Collected on the bushes of prickly rose, Komarov's raspberry, Daurian rose (*Rosa davurica* Pall.), rugosa rose, 04–28.IX.2015, 13–20.VI.2018, 04–20.IX.2019, total: 30 specimens (T.O. Markova).

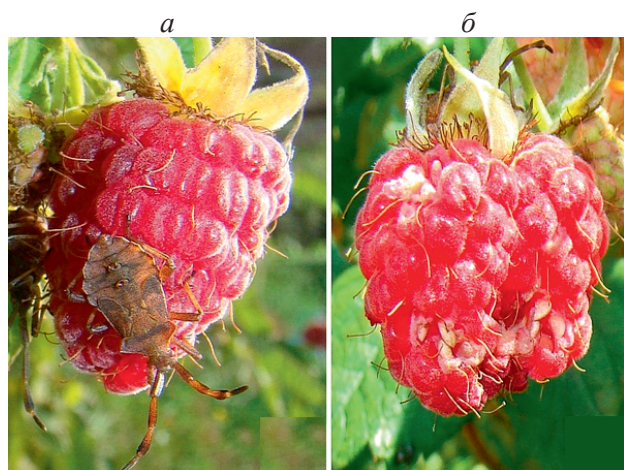
Feeding of *M. fuliginosa* on the mentioned plants has been confirmed in cages.

*C. m. orientalis* (see Fig. 3) has been observed in household plots located in the villages of Novomikhailovka (Chuguevsky District), Zarechnoye (Oktyabrsky District), Kaymanovka (Ussuriysk Urban District), and Pervomaiskoye (Khanka District). It was collected from common raspberry, remontant raspberry, and



**Рис. 2.** *Molipteryx fuliginosa* на окультуренных территориях и приусадебных участках (Приморский край): а – увядание апикальной части побегов *Rosa acicularis* в результате питания имаго, г. Уссурийск; б – имаго на листовой пластинке *R. rugosa*; в – усыхание плодов и апикальной части побегов *R. rugosa*, с. Каймановка

**Fig. 2.** *Molipteryx fuliginosa* in the cultivated areas and homestead plots (the Primorsky Territory): а – wilting of the apical part of *Rosa acicularis* shoots as a result of adult feeding, Ussuriysk; б – adults on the leaf lamina of *R. rugosa*; в – shriveling of fruits and apical part of shoots of *R. rugosa*, Kaimanovka village



**Рис. 3.** *Coreus marginatus orientalis* в агроценозах Приморского края:

*а* – питание личинки 5-го возраста на соплодии *Rubus idaeus*; *б* – усыхание костянок малины обыкновенной в результате высасывания соков личинками и имаго, с. Каймановка

**Fig. 3.** *Coreus marginatus orientalis* in agrocenoses of the Primorsky Territory:

*a* – feeding of V instar larvae on *Rubus idaeus* seedball; *б* – desiccation of common raspberry drupes as a result of sap sucking by larvae and adults, Kaimanovka village

dewberry bushes on 21.VII.2007, total: 10 specimens (Z.I. Limachko), 05.VI–20.VIII.2018, total: 13 specimens (A.S. Sakhnov, T.O. Markova), 02.VI–03.VII.2015, 25.VII.2016, 28–31.VII.2017, 08.VII–10.VIII.2018, 19.VI–29.VIII.2019, 19.VI–29.VIII.2022, total: 80 specimens (T.O. Markova, M.V. Maslov), 05–24.VII.2017, total: 10 specimens (A.V. Khovrina).

Feeding of *C. m. orientalis* on the mentioned plants has been confirmed in cages.

*M. fuliginosa* has been noted on prickly rose, rugosa rose, Daurian rose, Komarov's raspberry, and hawthorn raspberry, used in greening. In all cases, the bugs were observed feeding, sucking the juice from vegetative parts of the plants and fruit (see Fig. 2, *a, e*).

Common micro-stations and food sources for *C. m. orientalis* and *M. fuliginosa* in anthropo- and agrocenoses are common raspberry, remon- tant raspberry, and dewberry. Both species over- winter as adults. Mass copulation cases and for- mation of congregations of 7–10 individuals on raspberry and blackberry in agrocenoses were

noted during the appearance of young shoots and budding. Females of *M. fuliginosa* lay eggs on raspberry leaf blades and stems, while *C. m. orientalis* also lays directly on the fruit. Larvae of the 2nd–5th instar migrate to the upper parts of the plants, sucking juices from young shoots and inflorescences. As a result, normal develop- ment of laminas is disrupted, the growth point is suppressed, the apical part of the shoots wilts, and buds fall off. *M. fuliginosa* in larval and adult stages mainly feeds on vegetative parts of the plant, while *C. m. orientalis* prefers genera- tive organs, particularly the fruit, infesting them before they soften and reach physiological ripe- ness (see Fig. 3).

To combat these pests, gardeners are advised to collect copulating insects from garden plants to reduce the number of eggs laid on the leaves and stems, and to treat the plants in the second ten-day period of June to kill early-stage larvae. Specialists recommend such control measures as eliminating weeds during the raspberry growing season, cleaning up fallen leaves in autumn, and treating with Fitoverm, EC, and Fufanon, EC<sup>6</sup>.

## CONCLUSION

The conducted studies have shown that since 2012, the representatives of two species of bugs from the Coreidae family – the sorrel bug *C. m. orientalis* and the alien from Southeast Asia *M. fuliginosa* – have become serious pests in the anthropo- and agrocenoses of the Russian Far East. On cultivated plants (common raspberry, dewberry, and their remon- tant varieties), these insects congregate, mate, and lay eggs.

As a result of *M. fuliginosa*'s settlement, nor- mal development of laminas is disrupted, the growth point is suppressed, the apical part of the shoots wilts. *C. m. orientalis* on raspberry bush- es prefer the apical parts of the shoots from the fruit setting stage to full ripening. When the bugs feed on raspberry fruits, it results in damage to the receptacle and desiccation of the drupelets.

Thus, both species negatively affect the phys- iology of cultivated Rosaceae in agrocenoses. Further observations of these species in the Pri- morsky Territory are necessary, and they should

<sup>6</sup>Gardens and vegetable gardens [Electronic resource]. 2013, 1 Aug., N 46. URL: [https://vladnews.ru/ev/sad/46/2500/primore\\_obyavilsya](https://vladnews.ru/ev/sad/46/2500/primore_obyavilsya).

be included in the list of potential pests of cultivated Rosaceae.

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#### ИНФОРМАЦИЯ ОБ АВТОРАХ

✉ **Маркова Т.О.**, кандидат биологических наук, старший научный сотрудник; **адрес для переписки:** Россия, 690022, г. Владивосток, пр. 100-летия Владивостока, 159; e-mail: martania@mail.ru

**Маслов М.В.**, кандидат биологических наук, старший научный сотрудник

#### AUTHOR INFORMATION

✉ **Tatyana O. Markova**, Candidate of Science in Biology, Senior Researcher; **address:** 159, 100-th anniversary of Vladivostok ave., Vladivostok, 690022, Russia; e-mail: martania@mail.ru

**Mikhail V. Maslov**, Candidate of Science in Biology, Senior Researcher

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## ВЛИЯНИЕ ОРГАНОМИНЕРАЛЬНОЙ КОРМОВОЙ ДОБАВКИ НА ПРОДУКТИВНОСТЬ И БИОХИМИЧЕСКИЕ ПОКАЗАТЕЛИ ЦЫПЛЯТ-БРОЙЛЕРОВ

✉ Иванищева А.П., Сизова Е.А., Камирова А.М., Мусабаева Л.Л.

*Федеральный научный центр биологических систем  
и агротехнологий Российской академии наук*

Оренбург, Россия

✉ e-mail: nessi255@mail.ru

Представлены результаты исследований эффективности поликомпонентных кормовых добавок на основе растительных веществ как альтернативы антибиотикам. Отмечено, что пребиотики улучшают здоровье и продуктивность животных и повышают эффективность производственного процесса. При этом отдельное скармливание пребиотиков менее эффективно по сравнению с применением комплекса веществ разного функционального назначения. Проведена оценка влияния органоминеральной кормовой добавки с лактулозой и без нее на морфо-биохимический состав крови, переваримость компонентов рациона и продуктивность цыплят-бройлеров. Для опыта сформированы три группы ( $n = 90$ ): 1-я опытная группа дополнительно к основному рациону получала комплексную органоминеральную кормовую добавку начиная с 7-суточного возраста до конца эксперимента (42-суточный возраст); 2-я – ту же добавку, лишённую лактулозы. Контрольная группа получала стандартный основной рацион. Установлено положительное влияние органоминеральной добавки на продуктивные показатели цыплят-бройлеров, в большей степени в присутствии лактулозы. Содержание альбумина и общего белка в крови цыплят-бройлеров положительно коррелировало со скоростью роста у животных. Более высокая концентрация этих показателей в группе с лактулозой может способствовать усилению скорости роста у подопытных животных. В 1-й опытной группе отмечено увеличение прироста живой массы к концу исследования на 17,9%, тогда как во 2-й группе он составлял 10,8%. Также при рационе, содержащем лактулозу, показатели переваримости питательных веществ повысились к концу исследований на 3,8–4,7% по сравнению с контролем. Использование в кормлении цыплят-бройлеров органоминеральной кормовой добавки является перспективным подходом.

**Ключевые слова:** органоминеральная добавка, лактулоза, морфо-биохимические показатели, цыплята-бройлеры, поликомпонентная добавка

## INFLUENCE OF THE ORGANOMINERAL FEED ADDITIVE ON PRODUCTIVITY AND BIOCHEMICAL PARAMETERS OF BROILER CHICKENS

✉ Ivanishcheva A.P., Sizova E.A., Kamirova A.M., Musabayeva L.L.

*Federal Research Centre of Biological Systems and Agrotechnologies  
of the Russian Academy of Sciences*

Orenburg, Russia

✉ e-mail: nessi255@mail.ru

The results of research on the effectiveness of multicomponent feed additives based on plant substances as an alternative to antibiotics are presented. It has been observed that prebiotics improve animal health and productivity and increase the efficiency of the production process. At the same time, separate feeding of prebiotics is less effective compared to the use of a complex of substances

of different functional purpose. Effect of the organomineral feed additive with and without lactulose on morpho-biochemical composition of blood, digestibility of diet components and productivity of broiler chickens has been evaluated. Three groups ( $n = 90$ ) were formed for the experiment: the 1st experimental group in addition to the main diet received a complex organomineral feed additive, starting from 7-day-old until the end of the experiment (42-day-old); the 2nd group received the same additive without lactulose. The control group received the standard basic diet. The positive effect of organomineral additive on the productive parameters of broiler chickens was established, to a greater extent in the presence of lactulose. The content of albumin and total protein in the blood of broiler chickens was positively correlated with the growth rate in the animals. Higher concentration of these indices in the group with lactulose may contribute to enhanced growth rate in the experimental animals. The 1st experimental group showed an increase in the live weight gain by the end of the study by 17.9%, while in the 2nd group it was 10.8%. Also, with the diet containing lactulose, the indices of nutrient digestibility increased by the end of the study by 3.8–4.7% compared to the control. The use of the organomineral feed additive in the feeding of broiler chickens is a promising approach.

**Keywords:** organomineral additive, lactulose, morpho-biochemical parameters, broiler chickens, multicomponent additive

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**Конфликт интересов**

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**Conflict of interest**

The authors declare no conflict of interest.

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## INTRODUCTION

Currently, poultry product manufacturers are showing great interest in “natural” additives due to the ineffectiveness of antimicrobial agents and the ban on the use of antibiotics in animal feed. The search for safe and acceptable components and substances, as well as their combinations for use in feeding, is a pressing issue. Useful components of the diet in general, and as an alternative to antibiotics in particular, can include probiotics, prebiotics, herbs, and spices based on plant substances. Thanks to their multifunctional action, prebiotics improve animal health and productivity and increase the efficiency of the production process. However, feeding prebiotics separately is less effective compared to a complex of substances with different functional purposes. These can include macro- and microelements, metabolic agents, amino acids,

etc. Diets should contain the right balance of essential nutrients needed to meet the needs of poultry at various stages of production [1]. Lactulose can serve as a representative of prebiotic substances. Its main function as a prebiotic is to protect the intestinal microbial community. Under the influence of lactulose, the number of bifidobacteria and lactobacilli increases, while the number of clostridia, salmonella, or E. coli in the gastrointestinal tract decreases.

One way to enhance the activity of the nutrients, including trace elements, is to transform them into small-sized, particularly ultra-dispersed particles (UDPs), to increase their physicochemical activity and bioavailability for the living organism [2]. Porous ultra-dispersed silicon has found wide application in various fields of biology, medicine, and agriculture due to its unique biocompatibility, potential surface mod-

ification, and low toxicity. Also, UDPs of Si are of particular interest for use in feeding agricultural animals and poultry, as the main function of silicon is to participate in intermediary metabolism as a catalyst, as an element of connection, ensuring the normal flow of vital mechanisms [3]. Thus, a polycomponent organomineral feed additive can improve productive qualities and positively affect the health of broiler chickens.

The purpose of the study is to assess the effect of an organomineral feed additive with and without lactulose on the morpho-biochemical composition of blood, digestibility of diet components, and productivity of broiler chickens.

## MATERIAL AND METHODS

The research was conducted at the Center for Collective Use of Biological Systems and Agrotechnologies of the Russian Academy of Sciences (CCU BSA RAS<sup>1</sup>) on broiler chickens of the “Arbor Acres” cross.

To achieve the set goal, three groups ( $n = 90$ ) were formed: the 1st experimental group, in addition to the basic diet (BD), received a complex organomineral feed additive (40.81% lactulose, 28.57% arginine, 26.54% silicon (ultra-dispersed SiO<sub>2</sub> particles), 4.08% succinic acid) from the 7th day of age until the end of the experiment (42 days old); the 2nd group received the same additive but without lactulose. The control group was kept on the BD recommended by the All-Russian Research and Technological Institute of Poultry (VNIITIP)<sup>2</sup>. Ultra-dispersed silicon dioxide is a white amorphous powdery substance without a specific odor.

The mass fraction of silicon is at least 99.8% by weight, chlorine is not more than 0.2%, hydrodynamic diameter is  $388 \pm 117$  nm, specific surface area is 109 m<sup>2</sup>/g, Z-potential is  $-27 \pm 0.1$  mV (produced by “Plazmatern” LLC, Moscow, by plasma chemical synthesis method). Lactulose is a synthetic disaccharide consisting of two sugar molecules – fructose and galactose, linked together by a  $\beta$ -1,4-glycosidic bond, also belonging to the class of oligosaccharides, sub-

class of disaccharides. It is a white crystalline substance without odor, well soluble in water (produced by “Parusnik” LLC, St. Petersburg, by chemical isomerization of lactose in an alkaline environment). Slaughter was carried out at the age of 21 and 42 days.

Morphological blood parameters were determined using the DF-50 Vet automatic analyzer (Shenzhen Dymind Biotechnology Co., China). Biochemical analysis of blood serum was conducted on the CS-T 240 automatic biochemical analyzer (Dirui Industrial Co. Ltd., China) using commercial biochemical kits for veterinary medicine DiaVetTest (Russia). During the balance physiological experiments, the digestibility of the nutritional components of the diet was established according to VNIITIP methods. Based on the results of daily records of the mass of excrement, calculations were made to determine the loss of substances and the amount of feed assimilated. Statistical analysis was carried out using the Microsoft Excel program (Microsoft, USA). Data are presented as: mean value ( $M$ )  $\pm$  standard error of the mean ( $m$ ). The significance of differences was determined by the Student’s t-test. Results were considered significant at  $p \leq 0.05$ .

## RESULTS AND DISCUSSION

The results of this study indicate a positive effect of the organomineral additive on the productive parameters of broiler chickens, more significantly in the presence of lactulose (see Fig. 1). Experimental groups surpassed the control group in terms of live weight. The maximum difference was observed when feeding the additive containing lactulose. For instance, by the 14th day, the live weight of broiler chickens in the 1st group increased by 18.6%, and in the 2nd group by 14.5% compared to the control. By the middle of the study, the weight of the birds in both groups was the same, differing from the control by 14.6%.

The difference in live weight increase on the 35th and 42nd days was 10.7% and 17.9% in the

<sup>1</sup>URL: <http://цкп-бст.рф>

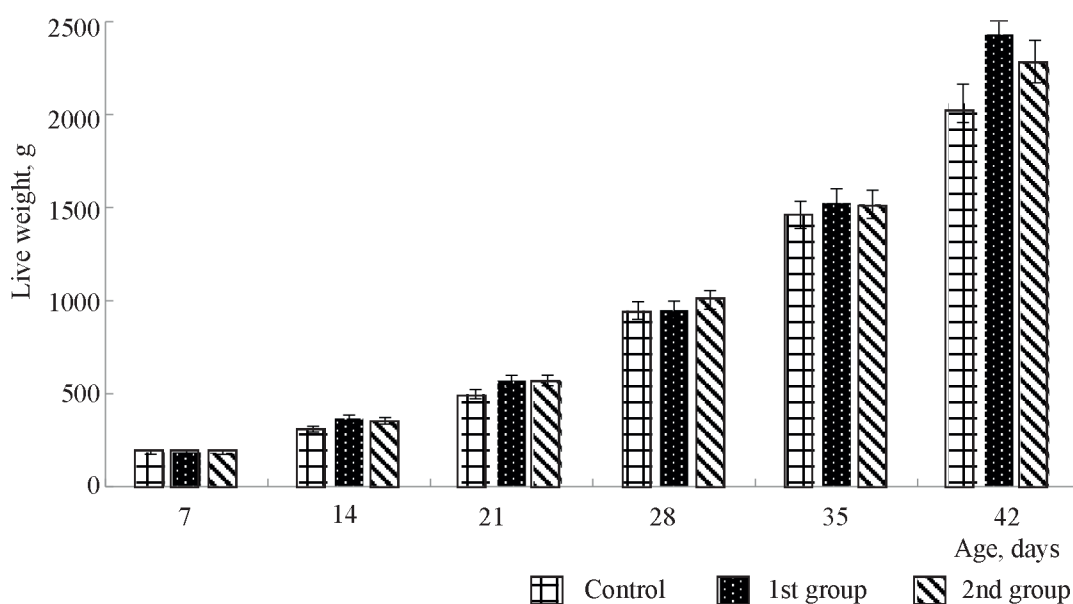
<sup>2</sup>Fisinin V.I., Egorov I.A. Modern approaches to feeding high-yielding poultry // Poultry and poultry products. 2015, N 3, pp. 27–29.

1st group, and 4.4% and 10.8% in the 2nd group, respectively, compared to the control. The enhanced growth of the broilers receiving lactulose may be related to increased feed consumption and improved morphofunctional components of the intestine. The use of the organomineral diet provides positive changes in the intestinal microflora. The prebiotic feed additive promotes better digestion of food while simultaneously increasing the digestibility of nutrients. For example, at 21 days old, the digestibility of crude fat and protein increased by 8.0% and 1.1% respectively in the 1st experimental group compared to the control (see Fig. 2). The digestibility of nutrients in diets supplemented with lactulose-containing additives may be due to improved intestinal conditions.

Digestibility of nitrogen-free extractive substances increased in both groups, with the maximum effect in the 1st group (2.8%). However, the digestibility of carbohydrates, in contrast, increased most significantly in the 2nd group (3.6%) compared to the control. Studies show that substances of prebiotic nature (inulin, oligofructose, lactulose) can stimulate *Lactobacillus* spp. and *Bifidobacterium* spp. as representatives of 'beneficial' bacterial species, thereby improving the intestinal environment and optimizing the processes of digestion and absorption of nutrients

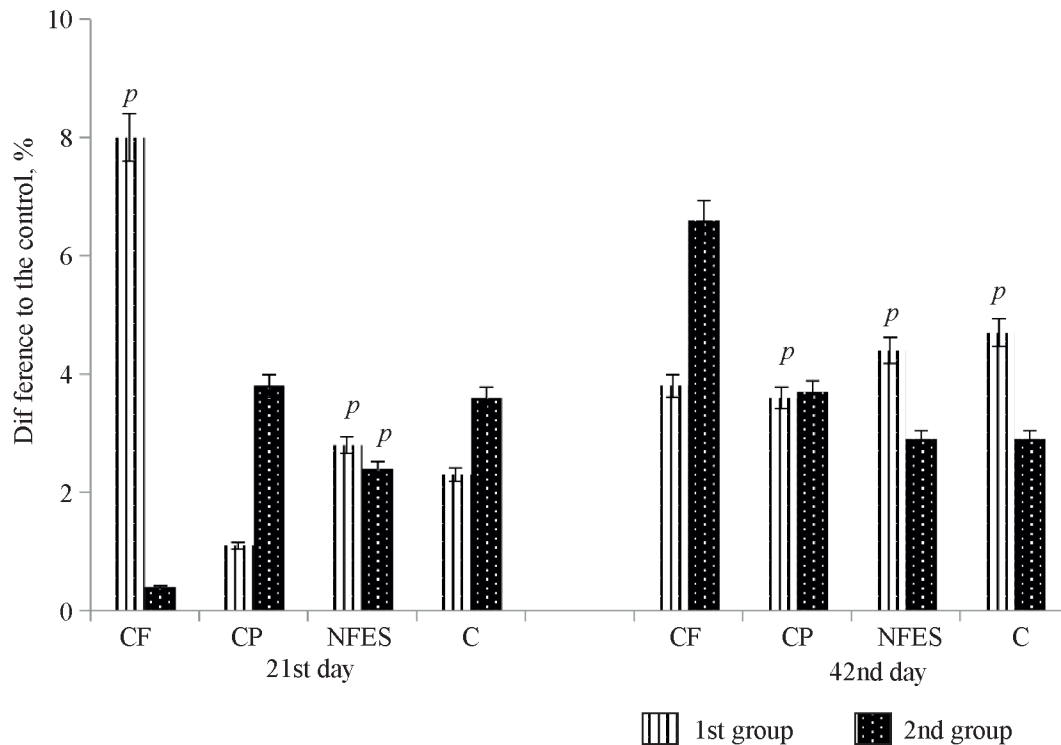
[4]. The group of soluble fibers includes oligosaccharides that function as prebiotics, positively modulating the intestinal microbiota [5]. They are the main substrates for the growth of intestinal microorganisms. Their fermentation leads to the acidification of the contents of the colon and the formation of short-chain fatty acids, which serve as 'fuel' in various tissues and may play a role in regulating cellular processes. By the end of the study (42 days), there were changes in the digestibility of all nutrients in the feed (see Fig. 2). For instance, in the 1st experimental group, the digestibility of crude fat increased by 3.8%, crude protein by 3.6%, metabolizable energy by 4.4%, and carbohydrates by 4.7% compared to the control. A similar effect was observed in the 2nd experimental group: digestibility of metabolizable energy and carbohydrates increased by 2.9%, crude fat, and protein by 6.6% and 3.7% respectively compared to the control.

Analysis of the blood morphological composition showed that the use of feed additives did not significantly change the concentration of erythrocytes. For example, its level increased by 5.5% in both experimental groups on the 21st day compared to the control (see Table 1). It's possible that the components of the feed additive indirectly contribute to the assimilation of the nutrients necessary for the synthesis of erythro-



**Рис. 1.** Динамика живой массы цыплят-бройлеров «Арбор Айкрес» (опыт в условиях вивария,  $M \pm m$ ,  $n = 30$ ).  
**Fig. 1.** Live weight dynamics of broiler chickens “Arbor Aykres” (experiment in vivarium conditions,  $M \pm m$ ,  $n = 30$ )





**Рис. 2.** Разница переваримости питательных веществ в возрасте 21 и 42 сут цыплят-бройлеров «Арбор Айкрес» относительно контроля (опыт в условиях вивария,  $M \pm m$ ,  $n = 30$ ), % (СЖ – сырой жир, СП – сырой протеин, БЭВ – безазотистые экстрактивные вещества; У – углеводы)  
Примечание.  $p$  – здесь и на рис. 3–5 – достоверная разница опытных групп с контрольной группой ( $p \leq 0,05$ ).

**Fig. 2.** The difference in the digestibility of nutrients at the age of 21 and 42 days of “Arbor Aykres” broiler chickens relative to the control (experience in vivarium conditions,  $M \pm m$ ,  $n = 30$ ), % (CF – crude fat, CP – crude protein, NFES – nitrogen-free extractive substances; C – carbohydrates)

Note.  $p$  – here and in Figs. 3–5 – reliable difference of the experimental groups with the control group ( $p \leq 0.05$ ).

cytes [6]. However, this same indicator on the 42nd day of the experiment decreased by 5% ( $p \leq 0.05$ ) in the 2nd experimental group compared to the control. The hemoglobin level also increased by the 21st day in all experimental groups: 1st group by 2.6% ( $p \leq 0.05$ ), 2nd group by 3.9% ( $p \leq 0.05$ ) compared to the control. By the end of the experiment, it was within the normal range.

The concentration of leukocytes on the 42nd day of the experiment, when lactulose was added (1st group), remained unchanged, while its exclusion from the diet (2nd group) led to a decrease by 8.5% compared to the control. Changes in their concentration indicate a low number of pathogens, leading to a reduction in the production of antibodies to fight them [7].

The study of biochemical indicators of blood serum in the experiment shows fluctuations in values within physiological norms. It was not

ed that adding the organomineral feed additive (1st group) affected the concentration of serum protein and albumin, increasing them by 1.48% and 3.5% (42nd day) respectively compared to the control.

Excluding lactulose from the feed additive composition (2nd experimental group) reduces the protein concentration by 1.29% on the 21st day and by 4.4% ( $p \leq 0.05$ ) on the 42nd day of the experiment (see Fig. 3).

It is widely known that total serum protein can reflect protein deposition in the body and, therefore, the growth rate of farm animals.

The creatinine level (42 days) in the 1st experimental group increased by 20.59% ( $p \leq 0.05$ ), while in the 2nd experimental group, there were no changes compared to the control.

The studies showed that the use of the organomineral feed additive in the diet affects fat metabolism (see Fig. 4). Thus, on the 21st

**Табл. 1.** Морфологические показатели крови цыплят-бройлеров «Арбор Айкрес» в возрасте 21 и 42 сут (опыт в условиях вивария,  $M \pm m$ ,  $n = 30$ ).

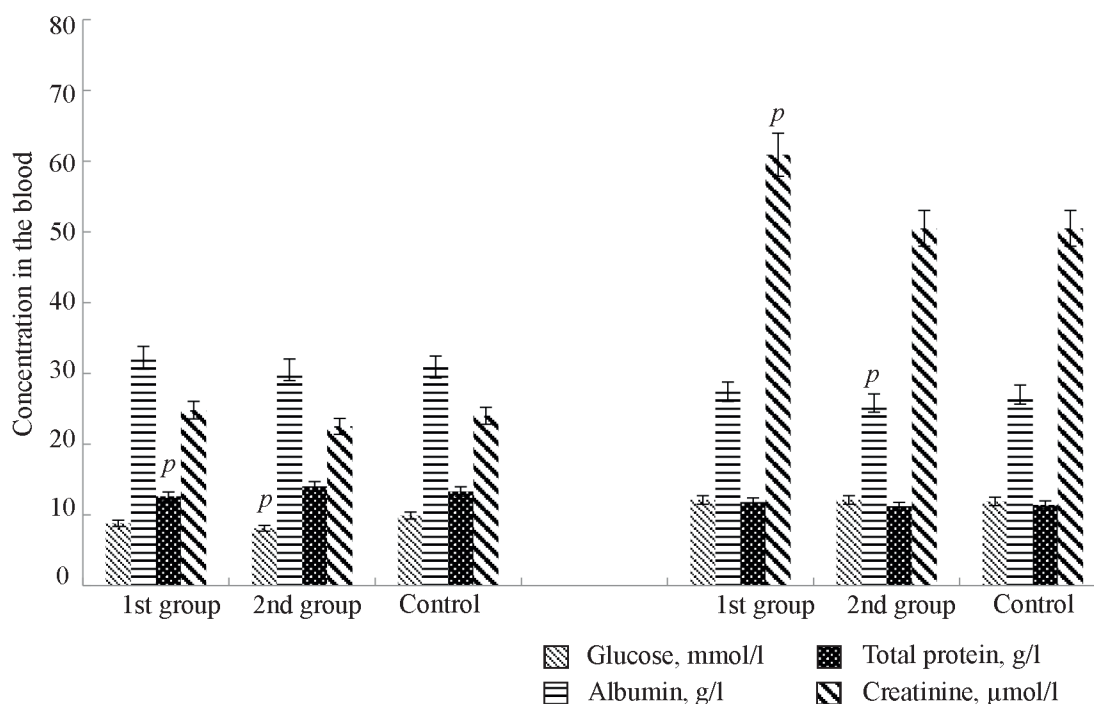
**Table 1.** Morphological blood parameters of the “Arbor Aykres” broiler chickens aged 21 and 42 days (experience in vivarium conditions,  $M \pm m$ ,  $n = 30$ )

Indicator	Control	1st group	2nd group
<i>21st day</i>			
Leukocytes, $10^9/l$	$40,6 \pm 2,59$	$42,9 \pm 3,07$	$40,0 \pm 2,88$
Erythrocytes, $10^{12}/l$	$1,8 \pm 0,03$	$1,9 \pm 0,04$	$1,9 \pm 0,08$
Hemoglobin, g/l	$103,6 \pm 1,20$	$106,3 \pm 2,98^*$	$107,6 \pm 1,88^*$
Hematocrit, %	$22,9 \pm 0,20$	$23,4 \pm 0,14^*$	$23,6 \pm 0,32^*$
<i>42 nd day</i>			
Leukocytes, $10^9/l$	$40,2 \pm 2,81$	$40,2 \pm 2,81$	$36,8 \pm 1,51$
Erythrocytes, $10^{12}/l$	$2,0 \pm 0,02$	$2,0 \pm 0,08$	$1,9 \pm 0,04$
Hemoglobin, g/l	$112,3 \pm 1,01$	$112,3 \pm 3,78$	$106,0 \pm 2,46$
Hematocrit, %	$24,6 \pm 0,78$	$24,6 \pm 0,78^*$	$23,4 \pm 0,51$

\* Significant difference between experimental groups and the control group ( $p \leq 0,05$ ).

day of the experiment, the concentration of triglycerides and cholesterol increased in all experimental groups, with the maximum effect when adding lactulose. On the 42nd day, the triglyceride level also increased in the 1st and 2nd groups by 33.3%, and cholesterol by 7.1% and 10.7% in the 1st and 2nd groups, respectively, compared to the control.

On the whole, as a number of studies show the growth of lactobacilli is enhanced against the background of probiotic intake, along with their ability to assimilate cholesterol in the presence of bile, thereby reducing its content in the blood of broiler chickens. Moreover, some microorganisms present in the gastrointestinal tract can use cholesterol for their own metabolism,



**Рис. 3.** Биохимические показатели крови цыплят-бройлеров «Арбор Айкрес» в возрасте 21 и 42 сут (опыт в условиях вивария,  $M \pm m$ ,  $n = 30$ )

**Fig. 3.** Biochemical blood parameters of the “Arbor Aykres” broiler chickens aged 21 and 42 days (experience in vivarium conditions,  $M \pm m$ ,  $n = 30$ )

thereby increasing the absorption of cholesterol. In our studies, there was a tendency to increase cholesterol and triglyceride levels in blood serum.

The use of soluble fibers, oligosaccharides functioning as prebiotics, positively affects the intestinal microbiota [8]. Their role in the development of hyperlipidemia has been proven. The use of prebiotics and probiotics has shown effectiveness in correcting such conditions [9]. Thus, the use of pre- and probiotics can alter blood lipid levels through several mechanisms: transformation of the microbiota and its metabolites, and reduced fat absorption.

The indicators of mineral metabolism changed during the experiment (see Fig. 5). The serum calcium content in the 1st group on the 21st day of the experiment decreased by 0.95%, but on the 42nd day, it increased by 3.7% compared to the control. The level of magnesium also increased on the 21st day (11.2%) and on the 42nd day (1.3%) of the study.

The phosphorus level decreased in the 1st experimental group throughout the study, while in the 2nd group, it first increased (on the 21st day) by 6.19% ( $p \leq 0.05$ ), then decreased (on the 42nd day) by 16.6% ( $p \leq 0.05$ ) relative to the control.

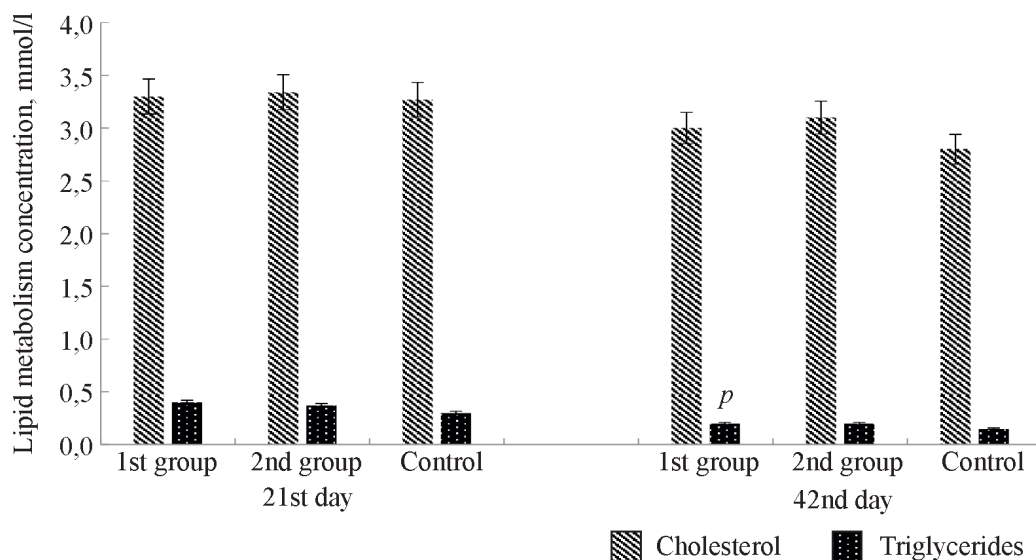
The iron content on the 21st day was higher in the 1st experimental group by 5.1%, and in the 2nd group, it was 22.4% lower than in the control. By the end of the experiment, a noticeable increase in iron levels was observed in all experimental groups – 5.9% and 49.01% respectively compared to the control group.

The analysis of production calculations for broiler production demonstrates the effectiveness of the proposed solution, particularly with the inclusion of the complex additive, which reduced feed consumption per 1 kg of weight gain by 12.4% (see Table 2). Including the organomineral complex in the broiler diet was accompanied by a reduction in the cost of 1 kg of meat by 2.55 rubles, leading to an increase in economic efficiency by 7%.

Thus, the conducted research proved the economic efficiency of including the organomineral complex in the diet of broiler chickens from 7 days of age. The identified combination is an effective source of increasing the productivity of broiler chickens.

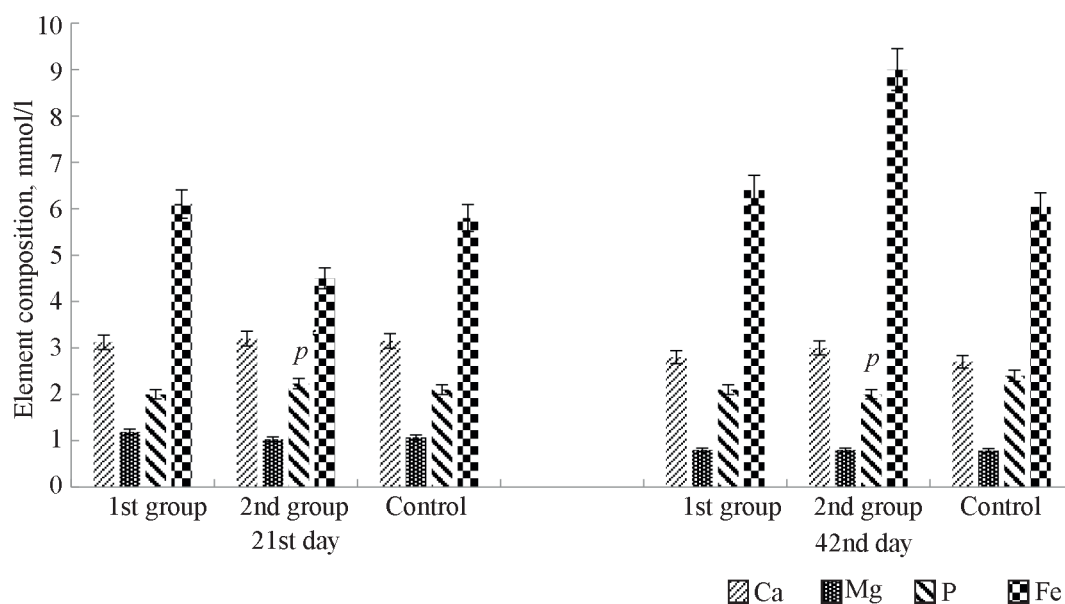
## CONCLUSION

Using organomineral feed additives in the feeding of broiler chickens is a promising approach. A diet containing lactulose positively



**Рис. 4.** Показатели липидного обмена сыворотки крови цыплят-бройлеров «Арбор Айкрес» в возрасте 21 и 42 сут (опыт в условиях вивария,  $M \pm m$ ,  $n = 30$ ), ммоль/л

**Fig. 4.** Some indicators of lipid metabolism of the “Arbor Aykres” broiler chickens blood serum aged 21 and 42 days (experience in vivarium conditions,  $M \pm m$ ,  $n = 30$ ), mmol/l



**Рис. 5.** Элементный состав сыворотки крови цыплят-бройлеров «Арбор Айкрес» в возрасте 21 и 42 сут (опыт в условиях вивария,  $M \pm m$ ,  $n = 30$ )

**Fig. 5.** Elemental composition of the “Arbor Aykres” broiler chickens blood serum of aged 21 and 42 days (experience in vivarium conditions,  $M \pm m$ ,  $n = 30$ )

**Табл. 2.** Экономическая эффективность по оценке продуктивного действия органоминерального комплекса

**Table 2.** Economic efficiency in assessing the productive action of the organomineral complex

Indicator	Option	
	Complex supplement	Complex supplement without lactulose
Number of poultry, heads	90	90
Average daily gain, g	45,2	45,2
Live weight of 1 head, g	2285	2431
Livability, %	100	100
Growing period, days	42	42
Feed consumption per 1 head, kg	2,54	2,9
Feed consumption per 1 kg of gain, kg	1,69	1,75
Slaughter weight:		
1 head, g	2285	2431
total, kg	205,2	218,7
Gutted carcass slaughter yield, %	70,9	77,8
Gutted carcass weight, g	1758	1893
Gutted meat received, kg	158,22	170,3
Production costs, total, rubles	14 118	15 574
Cost price of 1 kg of meat, rubles	74,7	77,25
Average selling price of 1 kg of meat with by-products, rubles	160	160
Total sales revenue, rubles	30 240	32 256
Profit from sales of meat and by-products, rubles	16 121	16 681
Profitability, %	14,1	7,1

affects the digestibility of nutrients, changing it by the end of the study in the range of 3.8–4.7% compared to the control. In the experiment, lactulose has a favorable effect on the health and productivity of poultry, enhancing weight gain by the end of the study by 17.9%.

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#### ИНФОРМАЦИЯ ОБ АВТОРАХ

✉ **Иванищева А.П.**, аспирант, специалист-техник, **адрес для переписки:** Россия, 460000, Оренбургская область, г. Оренбург, ул. 9 Января, 29; e-mail: nessi255@mail.ru

**Сизова Е.А.**, доктор биологических наук, руководитель центра «Нанотехнологии в сельском хозяйстве»

**Камирова А.М.**, кандидат биологических наук, научный сотрудник

**Мусабаева Л.Л.**, соискатель

#### AUTHOR INFORMATION

✉ **Anastasia P. Ivanishcheva**, Post-graduate Student, Specialist-technician; **address:** 29, January 9 St., Orenburg, Orenburg Region, 460000, Russia; e-mail: nessi255@mail.ru

**Elena A. Sizova**, Doctor of Science in Biology, Head of the Center «Nanotechnology in Agriculture»

**Ayna M. Kamirova**, Candidate of Science in Biology, Researcher

**Lera L. Musabaeva**, PhD student

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## ОСОБЕННОСТИ ПОДБОРА МИКРООРГАНИЗМОВ В СОСТАВ НОВОГО ПРОБИОТИЧЕСКОГО ПРЕПАРАТА ДЛЯ СЕЛЬСКОХОЗЯЙСТВЕННЫХ ЖИВОТНЫХ

✉ Функ И.А., Отт Е.Ф.

*Федеральный Алтайский научный центр агробиотехнологий*

Барнаул, Россия

✉ e-mail: funk.irishka@mail.ru

В настоящее время все большую популярность набирает идея здорового питания, что обуславливает интерес со стороны потребителей к экологически чистым и биологически полноценным продуктам животноводства. В связи с этим разработка пробиотических препаратов для сельскохозяйственных животных и определение эффективности их использования становятся актуальными задачами. Важными этапами при создании новых биопрепаратов являются изучение и подбор наиболее подходящих штаммов, так как пробиотический эффект входящих в состав закваски микроорганизмов определяется набором технологически ценных свойств, которыми они обладают. В процессе исследования были изучены технологически ценные свойства 12 штаммов лактобацилл и 15 штаммов пропионово-кислых бактерий из Сибирской коллекции микроорганизмов (лаборатория микробиологии молока и молочных продуктов, Сибирский научно-исследовательский институт сыроделия, ФГБНУ «Федеральный Алтайский научный центр агробиотехнологий») с целью включения их в состав нового пробиотического препарата для сельскохозяйственных животных. Установлено, что все рассматриваемые штаммы молочнокислых палочек активно сбраживали углеводы, входящие в состав растительных субстратов, а также сохраняли свою численность не ниже терапевтически значимого уровня в течение 60 сут. Пропионово-кислые бактерии продуцировали от  $0,48 \pm 0,01$  до  $0,64 \pm 0,06$  мкг/см<sup>3</sup> витамина В<sub>12</sub> и сохраняли свою жизнеспособность в количестве десятков миллионов клеток на протяжении 6 мес. В результате по наилучшему проявлению технологически ценных свойств было отобрано два штамма молочнокислых палочек (СКМ-673, СКМ-681) и три штамма пропионово-кислых бактерий (11<sub>1</sub>, 11<sub>2</sub>, 149) как перспективных для включения в состав нового пробиотического препарата для сельскохозяйственных животных.

**Ключевые слова:** пробиотические микроорганизмы, технологически ценные свойства, активность кислотообразования, ферментативная активность, сохранность

## FEATURES OF SELECTION OF MICROORGANISMS IN THE COMPOSITION OF A NEW PROBIOTIC DRUG FOR FARM ANIMALS

✉ Funk I.A., Ott E.F.

*Federal Altai Scientific Center of Agrobiotechnologies*

Barnaul, Russia

✉ e-mail: funk.irishka@mail.ru

Nowadays, the idea of healthy eating is gaining popularity, which makes consumers interested in environmentally friendly and biologically nutritious animal products. In this regard, the development of probiotic preparations for farm animals and determination of the effectiveness of their use become urgent tasks. Important stages in the creation of new biopreparations are the study and selection of the most suitable strains, as the probiotic effect of the microorganisms included in the inoculum is determined by a set of technologically valuable properties they possess. In the course of the study, technologically valuable properties of 12 strains of lactobacilli and 15 strains of propionic acid bacteria from the Siberian Collection of Microorganisms (Laboratory of Microbiology of Milk and Dairy Products, Siberian Research Institute of Cheesemaking, FSBSI "Federal Altai Scientific Center of Agrobiotechnologies") were studied in order to include them in the composition of a new probiotic preparation for farm animals. It was found that all the considered strains of lactic acid bacilli actively digested carbohydrates included in the composition of plant substrates, as well as maintained their abundance not below the therapeutically significant level for 60 days. Propionic acid bacteria produced  $0.48 \pm$

0.01 to  $0.64 \pm 0.06 \mu\text{g}/\text{cm}^3$  of vitamin B<sub>12</sub> and remained viable in numbers of tens of millions of cells for 6 months. As a result, two strains of lactic acid bacilli (SCM 673, SCM 681) and three strains of propionic acid bacteria (11<sub>1</sub>, 11<sub>2</sub>, 149) were selected according to the best manifestation of technologically valuable properties as promising for inclusion in the composition of a new probiotic preparation for farm animals.

**Keywords:** probiotic microorganisms, technologically valuable properties, acid formation activity, enzymatic activity, preservation

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#### Conflict of interest

The authors declare no conflict of interest.

## INTRODUCTION

In the Russian Federation, there is a justified increase in hygienic requirements for livestock products, leading to a growing demand for biologically active agents, particularly probiotics, considered as an alternative to feed antibiotics.

Numerous experiments conducted by domestic and foreign researchers have proven that the use of probiotics in the diet of agricultural animals promotes better digestion and assimilation of nutrients and biologically active substances, normalization of metabolic processes, and increased overall resistance of the organism. As a result of the complex action of probiotic preparations on the animal's body, the yield of agricultural products is increased and their quality is improved [1, 2].

The therapeutic properties of probiotic preparations are determined by the qualitative composition of the microflora and the quantitative content of microorganisms. Typically, microorganisms exhibiting probiotic properties belong to the genera *Bifidobacterium*, *Lactobacillus*, *Propionibacterium*, *Leuconostoc*, *Enterococcus*, *Escherichia*, *Pediococcus*, as well as yeasts *Saccharomyces*, which stabilize the microflora, restore its disturbed balance, and stimulate the immunological function of the mucous membrane of the gastrointestinal tract [3].

In turn, the probiotic effect of microorganisms is determined by the set of technologically valuable properties they possess, which is

important to consider when selecting probiotic strains for new biopreparations. It is considered that lactic acid and propionic acid bacteria are the most promising cultures for inclusion in probiotic preparations for agricultural animals. For instance, lactic acid bacteria, particularly lactobacilli, are capable of producing antibiotic-like substances and organic acids, which is the main mechanism of their antagonistic activity against conditionally pathogenic and pathogenic microflora of the gastrointestinal tract [4]. Propionic acid bacteria have unique biochemical properties. They can produce propionic and acetic acids, superoxide dismutase, catalase, as well as vitamin B<sub>12</sub>, which is very important for humans and animals [5, 6].

However, it is worth noting that the properties of lactobacilli and propionic acid bacteria mentioned above can manifest to a greater or lesser extent in different strains of microorganisms.

Thus, the selection and inclusion of probiotic microorganisms in the composition of biopreparations under development based on the study of their technologically valuable properties is an important stage in creating truly high-quality probiotics, which was the goal of this study.

To achieve this goal, the following tasks were set:

1. Study the acid-forming activity, enzymatic activity, and preservation of collection cultures of lactobacilli.



2. Evaluate the enzymatic activity of propionic acid bacteria, their ability to produce vitamin B<sub>12</sub>, and the dynamics of their population during storage.

3. Based on the analysis of the degree of manifestation of technologically valuable properties, select strains of propionic acid bacteria and lactobacilli for inclusion in the composition of a new biopreparation for agricultural animals.

## MATERIAL AND METHODS

To select strains of probiotic microorganisms for the composition of a biopreparation under development, 12 cultures of lactic acid rods (*Lactobacillus* spp.) and 15 cultures of propionic acid bacteria from the Siberian Collection of Microorganisms were studied from 2018 to 2022. The research was conducted at the laboratory of milk and dairy products microbiology and the laboratory of applied biotechnology of the Siberian Research Institute of Cheesemaking, part of the Federal Altai Scientific Center for Agrobiotechnology (Russia, Barnaul).

The main technologically valuable properties of the studied cultures were examined using standard microbiological and biochemical analysis methods. The active acidity of lactic acid rods was determined potentiometrically according to GOST R 53359–2009<sup>1</sup>. The ability to ferment carbohydrates, both in lactic acid rods and propionic acid bacteria, was qualitatively determined in the Geese liquid nutrient medium according to the generally accepted method. The results were assessed by the change in color of the nutrient medium with carbohydrates. The production of vitamin B<sub>12</sub> by propionic acid bacteria was determined quantitatively within the diffusion method and using the test organism *Escherichia coli* 113-3 DSM 1900 (FGNU “State Research Institute of Genetics and Selection of Industrial Microorganisms”)<sup>2</sup> [7]. The number of lactic acid rods and propionic

acid bacteria during storage was determined according to GOST 10444.11–2013<sup>3</sup> and GOST R 56139–2014<sup>4</sup>.

## RESULTS AND DISCUSSION

One of the positive properties of lactobacilli is the production of lactic acid and other organic acids, which helps to suppress foreign microflora in the gastrointestinal tract and create optimal conditions for the normal development of other representatives of symbiotic microflora [8].

Therefore, the study examined the acid-forming activity of 12 strains of *Lactobacillus plantarum*. The research results are presented in Table 1.

During the experiment, it was found that signs of growth were noted in all the studied strains after 8 hours of cultivation. The active acidity of the cultural fluid varied from  $5.68 \pm 0.24$  to  $4.09 \pm 0.11$  pH units. pH changes were observed during the first 4 days of cultivation. From the 5th to the 10th day, there was a slight change in active acidity. The maximum acid formation was at the level of  $3.77 \pm 0.19$  to  $3.12 \pm 0.27$  pH units.

Thus, among the 12 studied cultures of *L. plantarum*, the best acid formation was noted in 50% of the lactic acid rods strains (SKM-646, SKM-651, SKM-656, SKM-669, SKM-681, SKM-683).

When selecting lactobacilli for inclusion in probiotics for agricultural animals, preference is given to the strains that ferment carbohydrates present in plant substrates [9]. This property of microorganisms allows for more rational and efficient use of the feeds. Therefore, it was important to study *L. plantarum* lactic acid rods in terms of their ability to ferment carbohydrates contained in plant substrates. The data on the fermentative activity of lactobacilli are presented in Table 2.

The results presented in Table 2 show that all the studied strains of lactic acid rods possessed

<sup>1</sup>GOST R 53359-2009. Milk and milk products. Method for determination of pH. Moscow: Standardinform, 2009, 11 p.

<sup>2</sup>Egorova N.S. Practicum on microbiology: textbook. Moscow: Moscow State University Publishing House, 1976, 307 p.

<sup>3</sup>GOST 10444.11-2013 (ISO 15214:1998). Microbiology of food products and animal feed. Methods of detection and counting of mesophilic lactic acid microorganisms. Moscow: Standardinform, 2014, 22 p.

<sup>4</sup>GOST R 56139-2014. Functional food products. Methods of determination and counting of probiotic microorganisms. Moscow: Standardinform, 2015, 32 p.

**Табл. 1.** Активная кислотность исследуемых штаммов *L. plantarum* ( $\mu\text{pH} \pm \text{pH}$ )  
**Table 1.** Active acidity of the studied *L. plantarum* strains ( $\mu\text{pH} \pm \text{pH}$ )

No. n/a	Strain index	Indicators of active acidity during 10 days, units									
		4 h	8 h	1 day	2 days	3 days	4 days	5 days	7 days	10 days	
1	SKM-646	5,87 ± 0,27	5,38 ± 0,17	3,66 ± 0,11	3,41 ± 0,08	3,34 ± 0,05	3,28 ± 0,05	3,29 ± 0,12	3,29 ± 0,09	3,28 ± 0,15	
2	SKM-651	5,95 ± 0,19	5,53 ± 0,24	3,73 ± 0,03	3,34 ± 0,05	3,25 ± 0,03	3,17 ± 0,03	3,17 ± 0,03	3,14 ± 0,05	3,13 ± 0,08	
3	SKM-656	5,51 ± 0,03	4,46 ± 0,03	3,49 ± 0,05	3,33 ± 0,05	3,30 ± 0,05	3,29 ± 0,12	3,28 ± 0,05	3,27 ± 0,05	3,27 ± 0,03	
4	SKM-667	5,95 ± 0,24	5,51 ± 0,88	4,03 ± 0,24	3,74 ± 0,24	3,51 ± 0,03	3,52 ± 0,19	3,47 ± 0,13	3,48 ± 0,13	3,49 ± 0,05	
5	SKM-668	5,86 ± 0,21	5,33 ± 0,77	3,93 ± 0,24	3,58 ± 0,08	3,53 ± 0,29	3,44 ± 0,13	3,43 ± 0,27	3,43 ± 0,11	3,42 ± 0,08	
6	SKM-669	4,86 ± 0,03	4,09 ± 0,11	3,47 ± 0,03	3,36 ± 0,02	3,31 ± 0,02	3,31 ± 0,03	3,31 ± 0,03	3,30 ± 0,12	3,32 ± 0,02	
7	SKM-671	5,07 ± 0,55	4,43 ± 0,82	3,71 ± 0,55	3,44 ± 0,03	3,44 ± 0,03	3,45 ± 0,14	3,46 ± 0,02	3,44 ± 0,05	3,45 ± 0,10	
8	SKM-673	5,91 ± 0,08	5,68 ± 0,24	4,26 ± 0,88	3,68 ± 0,11	3,59 ± 0,38	3,58 ± 0,28	3,58 ± 0,45	3,59 ± 1,2	3,59 ± 0,4	
9	SKM-681	5,26 ± 0,12	4,22 ± 0,11	3,43 ± 0,05	3,3 ± 0,02	3,26 ± 0,12	3,25 ± 0,12	3,26 ± 0,12	3,25 ± 0,03	3,25 ± 0,05	
10	SKM-683	5,84 ± 0,05	5,0 ± 0,68	3,42 ± 0,03	3,22 ± 0,11	3,15 ± 0,27	3,13 ± 0,24	3,11 ± 0,24	3,10 ± 0,27	3,12 ± 0,27	
11	SKM-690	5,68 ± 0,93	5,34 ± 0,91	4,09 ± 0,23	3,82 ± 0,24	3,85 ± 0,27	3,75 ± 0,27	3,77 ± 0,29	3,76 ± 0,23	3,77 ± 0,19	
12	SKM-694	4,84 ± 0,30	4,14 ± 0,13	3,54 ± 0,03	3,48 ± 0,02	3,47 ± 0,03	3,48 ± 0,05	3,49 ± 0,03	3,47 ± 0,05	3,49 ± 0,06	

relatively high ability to ferment carbohydrates. Specifically, 66.7% of the strains fermented arabinose, 58.3% fermented starch, 33.3% fermented raffinose, and 100% fermented mannose, sucrose, and cellobiose. The highest fermentative activity was noted in the strains SKM-651, SKM-669, SKM-673, and SKM-681.

On the other hand, the study of the fermentative activity of propionic acid bacteria revealed that the investigated strains comparatively weakly fermented carbohydrates found in plant substrates, as they belong to the classical (lactic) types of propionic acid bacteria. For example, 33.3% of the strains fermented arabinose, 6.7% fermented starch, 66.7% fermented mannose, and 13.3% fermented raffinose, while none of the strains fermented sucrose and cellobiose. However, it's important to note that the relatively low fermentative activity of propionic acid bacteria in relation to carbohydrates found in plant substrates does not diminish the importance of including them in the composition of developed probiotics, as one of their most important properties is the ability to actively synthesize vitamin B<sub>12</sub>. This vitamin has high biological activity and performs several vital functions in the bodies of humans and animals. Animals do not synthesize vitamin B<sub>12</sub> in their tissues; it is produced only by the microflora of the gastrointestinal tract. The body's need for this vitamin is not always fully met by microbial synthesis alone, so additional quantities must be supplied from external sources [10]. This fact should be considered when selecting propionic acid bacteria for inclusion in biopreparations.

In the study, 15 strains of propionic acid bacteria were examined for their ability to produce vitamin B<sub>12</sub>. The results of the experiment are reflected in Table 3.

From Table 3, it is evident that all the studied strains of propionic acid bacteria have the capability to synthesize vitamin B<sub>12</sub>, justifying the rationale for including this group of microorganisms in the composition of probiotic preparations. The production of vitamin B<sub>12</sub> was at the level of 0.48 ± 0.01 to 0.64 ± 0.06 μg/cm<sup>3</sup>.

The content of probiotic microorganisms in biologically active preparations determines their therapeutic properties. Therefore, when devel-

**Табл. 2.** Ферментирование углеводов молочнокислыми палочками *L. plantarum*  
**Table 2.** Fermentation of carbohydrates with lactic acid sticks of *L. plantarum*

No. n/a	Strain index	Fermentable carbohydrates					
		arabinose	starch	mannose	raffinose	saccharose	cellobiose
1	SKM -646	+	+	+	-	+	+
2	SKM -651	-	+	+	+	+	+
3	SKM -656	-	+	+	-	+	+
4	SKM -667	+	-	+	-	+	+
5	SKM -668	-	+	+	-	+	+
6	SKM -669	+	+	+	+	+	+
7	SKM -671	+	-	+	-	+	+
8	SKM -673	+	-	+	+	+	+
9	SKM -681	+	-	+	+	+	+
10	SKM -683	-	+	+	-	+	+
11	SKM -690	+	+	+	-	+	+
12	SKM -694	+	-	+	-	+	+

Note. «+» - change of coloration of the nutrient medium (positive result); «-» - no change in the coloration of the nutrient medium (negative result).

oping biopreparations, it is important to consider the ability of microorganisms to maintain their numbers during storage.

The conducted research showed that the cell count of propionic acid bacteria remained at a level not lower than therapeutically significant (at least  $10^6$  CFU/cm<sup>3</sup>) for 6 months of storage, and after 180 days, it amounted to tens of millions of cells per 1 ml.

Similarly, all the studied strains of lactic acid rods also remained at a level not lower than  $1.0 \times 10^6$  CFU/cm<sup>3</sup> for 60 days of storage. The initial count of lactobacilli varied from  $7.21 \pm 0.20 \times 10^8$  to  $5.85 \pm 0.21 \times 10^8$  CFU/cm<sup>3</sup>. The number of lactic acid rods after 20 days of storage was in the hundreds of millions of cells per ml, and from the 30th to the 60th day, the count decreased to tens of millions of cells, and after 60 days, it was at the level of  $3.60 \pm 0.15 \times 10^7$  to  $1.56 \pm 0.11 \times 10^7$  CFU/cm<sup>3</sup>.

## CONCLUSION

In conclusion, the studied strains of lactic acid rods demonstrated high technologically valuable properties, as evidenced by active fermentation of carbohydrates found in plant substrates and the maintenance of viable bacterial cells for 60 days. Propionic acid bacteria exhibited comparatively low fermentative activity in relation to carbohydrates found in plant substrates, but they

**Табл. 3.** Продуцирование витамина B<sub>12</sub> пропионово-кислыми бактериями

**Table 3.** Production of vitamin B<sub>12</sub> by propionic acid bacteria

No. n/a	Strain index	Amount of vitamin B <sub>12</sub> , mkg/cm <sup>3</sup>
1	AM-1 <sup>2</sup>	0,48 ± 0,01
2	AM-1 <sup>1</sup>	0,50 ± 0,02
3	11 <sub>1</sub>	0,60 ± 0,01
4	149	0,58 ± 0,05
5	X <sub>3</sub>	0,57 ± 0,03
6	11 <sub>2</sub>	0,64 ± 0,06
7	B-3	0,37 ± 0,01
8	B-2	0,53 ± 0,07
9	M <sub>1</sub> <sup>2</sup>	0,56 ± 0,05
10	BM-3 <sup>2</sup>	0,50 ± 0,03
11	BM-2 <sup>1</sup>	0,51 ± 0,02
12	AM-3 <sup>1</sup>	0,45 ± 0,04
13	AM-3 <sup>2</sup>	0,45 ± 0,02
14	12 <sup>1</sup>	0,57 ± 0,06
15	12 <sup>2</sup>	0,50 ± 0,02

showed high biological activity: they could produce from 0.48 to 0.64 µg/cm<sup>3</sup> of vitamin B<sub>12</sub> and maintain the number of bacterial cells at a level not lower than therapeutically significant for 180 days. Considering the technologically valuable properties of lactic acid rods and propionic acid bacteria, the most promising cultures for inclusion in the composition of the developed

biopreparation for agricultural animals are two strains of lactobacilli (SKM-673, SKM-681) and three strains of propionic acid bacteria (11<sub>1</sub>, 11<sub>2</sub>, and 149).

## СПИСОК ЛИТЕРАТУРЫ

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#### ИНФОРМАЦИЯ ОБ АВТОРАХ

✉ **Функ И.А.**, кандидат сельскохозяйственных наук, старший научный сотрудник; **адрес для переписки:** Россия, 656910, г. Барнаул, Научный городок, 35; e-mail: funk.irishka@mail.ru

**Отт Е.Ф.**, кандидат биологических наук, ведущий научный сотрудник

#### AUTHOR INFORMATION

✉ **Irina A. Funk**, Candidate of Science in Agriculture, Senior Researcher; **address:** 35, Nauchnyy gorodok, Barnaul, 656910, Russia; e-mail: funk.irishka@mail.ru

**Ekaterina F. Ott**, Candidate of Science in Biology, Lead Researcher

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## МЕТОДЫ ПРОФИЛАКТИКИ КЛОСТРИДИАЛЬНОЙ ИНФЕКЦИИ КРУПНОГО РОГАТОГО СКОТА НА ТЕРРИТОРИИ РОССИЙСКОЙ ФЕДЕРАЦИИ

**Безбородова Н.А., Томских О.Г., (✉)Кожуховская В.В., Порываева А.П., Сажаев И.М.**  
*Уральский федеральный аграрный научно-исследовательский центр Уральского отделения  
Российской академии наук*  
Екатеринбург, Россия  
(✉)e-mail: Tetramegon@yandex.ru

Представлены обзор опубликованных материалов зарубежных и отечественных ученых, анализ результатов собственных исследований по проблеме клостридиозов крупного рогатого скота. Отражена современная этиологическая структура клостридиозов на территории Российской Федерации в соответствии с данными Центра ветеринарии (Москва). Описан видовой состав бактерий рода *Clostridium*, вызывающих заболевания у крупного рогатого скота, более чем в 17 регионах нашей страны. Приведены данные о видовом разнообразии клостридий и вакцинации против ЭМКАР поголовья крупного рогатого скота в Уральском регионе. Представлена актуальная информация об иммунобиологических препаратах против клостридиозов крупного рогатого скота, официально внесенных в Государственный реестр лекарственных средств для ветеринарного применения в России. Освещены вопросы иммуногенности вакцин и эффективности их применения на поголовье крупного рогатого скота в зависимости от различных факторов. Приведены сравнительные данные применения отечественных и зарубежных вакцин в ветеринарной практике на сельхозпредприятиях, достоинства и недостатки используемых препаратов. Рассмотрены современные российские иммунобиологические лекарственные средства против клостридиозов крупного рогатого скота. Кратко описаны основные меры профилактики по каждому инфекционному заболеванию, вызываемому клостридиями.

**Ключевые слова:** вакцинация, иммунизация, вакцина, специфическая профилактика, клостридии, анаэробы, крупный рогатый скот, *Clostridium*, сельскохозяйственные организации

## PREVENTION METHODS FOR CLOSTRIDIAL INFECTION OF CATTLE ON THE TERRITORY OF THE RUSSIAN FEDERATION

**Bezborodova N.A., Tomskikh O.G., (✉)Kozhuhovskaya V.V., Poryvaeva A.P., Sazhaev I.M.**  
*Ural Federal Agrarian Scientific Research Centre – Ural Branch of the Russian Academy of Sciences*  
Ekaterinburg, Russia  
(✉)e-mail: Tetramegon@yandex.ru

This article presents a review of the literature data of foreign and domestic scientists, as well as an analysis of the results of our own research on the problem of clostridial infections in cattle. This paper provides insight into current etiological structure of clostridiosis in the Russian Federation in accordance with the data of the Federal State Budgetary Institution “Center for Veterinary Medicine”. The species composition of bacteria of the genus *Clostridium*, which causes diseases in cattle in more than 17 regions of Russia, is described. Moreover, data on the *Clostridia* species diversity in the Ural region and information on vaccination against blackleg of cattle bred in the Sverdlovsk region are presented. The issues of immunogenicity of vaccines and the effectiveness of their use in cattle, depending on various factors, are highlighted. Up-to-date information on immunobiological preparations against clostridial diseases in cattle officially included in the register of medicinal products of the Russian Federation is presented. The advantages and disadvantages of domestic and foreign vaccines used in veterinary practice are analyzed. A review of current data in the field of new developments of Russian immunobiological agents against clostridial infections in cattle is presented. Comparative data on the use of domestic and foreign vaccines in veterinary practice at agricultural organizations are given. In a brief form, the main preventive measures for each infectious disease caused by clostridia are described.

**Keywords:** vaccination, immunization, vaccine, specific prophylaxis, clostridia, anaerobes, cattle, *Clostridium*, agricultural organizations

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#### Conflict of interest

The authors declare no conflict of interest.

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Clostridiosis are diseases of warm-blooded animals caused by anaerobic spore-forming microorganisms of the genus *Clostridium*. This genus consists of 250 species of bacteria. The exotoxins produced by clostridia cause damage to the gastrointestinal tract, soft tissues, and nervous system of varying severity [1, 2]. For cattle, the following clostridial infections are epizootically and diagnostically significant: blackleg, anaerobic enterotoxemia, malignant edema, tetanus, hemorrhagic enteritis, necrotic hepatitis, and enteritis [3]. These infections are caused by pathogenic anaerobic bacteria *C. perfringens*, *C. difficile*, *C. tetani*, *C. chauvoei*, *C. septicum*, *C. novyi* [3–5].

According to the Veterinary Center (Moscow), individual cases of clostridiosis in animals are recorded annually [3]. In cattle herds, clostridial diseases such as malignant edema and anaerobic enterotoxemia are more commonly diagnosed, while blackleg is less common. Tetanus has not been detected in the last 10 years. According to monitoring data from 2007 to 2017, the incidence of clostridial infections in cattle has consistently decreased. For example, the number of cases of anaerobic enterotoxemia decreased by 88.3%, blackleg by 95.2%, and malignant edema by 70.0% [6]. It should be

noted that official data do not always reflect the current situation in terms of disease incidence<sup>1</sup> [3, 5].

A.V. Kapustin and colleagues conducted a survey of 32 farms from 16 regions of the Russian Federation. They studied 2,913 samples of biomaterial from 983 cows and 119 calves of various ages<sup>2</sup> [3, 6]. In the analyzed samples, *C. septicum* (34.5%), *C. perfringens* type A (23.25%), and *C. perfringens* type C (14.25%) were most frequently identified. In cases of monoinfection, *C. perfringens* type D and *C. sordellii* (6.5%) and *C. novyi* (2.5%) were identified. *C. tetani*, *C. sporogenes*, *C. bifermentans*, *C. baratii*, *C. tertiu*, *C. hastiforme*, *C. difficile*, *C. innocuum*, *C. histolyticum* were found individually (see footnote 2).

Russian scientists also identified the species diversity of *Clostridium* pathogens in Moscow, Tyumen, Ryazan, Kursk, Irkutsk, Nizhny Novgorod, Kirov, Tver, Belgorod, Pskov, Saratov, Samara, Chelyabinsk regions, and the Republic of Mordovia: isolates of *C. perfringens*, *C. septicum*, *C. sporogenes* occupy a dominant position, while *C. difficile*, *C. butyricum*, *C. bifermentans* are found individually [4, 7].

According to the Department of Veterinary Medicine of the Sverdlovsk region, during ep-

<sup>1</sup>Glotova T.I., Terentyeva T.E., Glotov A.G. Pathogens and age susceptibility of cattle to clostridiosis // *Siberian Herald of Agricultural Science*, 2017, vol. 47, N 1 (254), pp. 90–96.

<sup>2</sup>Dementieva M.S., Krysenko Y.G. Comparative economic efficiency of immunoprophylaxis schemes of bovine clostridiosis // *Scientific developments and innovations in solving strategic problems of agroindustrial complex: Proceedings of the International scientific-practical conference (Izhevsk, February 15-18, 2022)*. Izhevsk: Izhevsk State Agricultural Academy Publishing House, 2022, pp. 149–153.

idemiological monitoring from 2007 to 2021, ten outbreaks of blackleg were registered on five agricultural enterprises located in the Krasnoufimsky, Belyarsky, Artinsky, and Nevyansky districts. Additionally, during the same period, 13 cases of malignant edema were recorded on four agricultural enterprises in the Novouralsky urban district, Taborinsky, Krasnoufimsky, and Achitsky districts.

From 2020 to 2022, using molecular genetic methods, we conducted diagnostic studies of biological samples from cattle at 12 livestock enterprises in the Sverdlovsk region. In the samples of feces, homogenates from pathological material of deceased calves and cows, and milk samples, DNA genomes of *C. difficile* and *C. perfringens* were detected in 76.2% of cases. The total proportion of *C. difficile* genome detection in biological samples was 56.2%, and that of *C. perfringens* genome was 45.0%, with both genomes detected simultaneously in 25.0% of cases. It was established that 48.0% of the detected *C. difficile* were toxigenic, and 16.2% did not have a complete pathogenic locus. The main toxintype of *C. difficile* was CDT (41.0%), toxintype B was found in 6.0% of clostridia, and toxintype A in 1.0%. In one case, genes of virulence responsible for the production of toxins A, B, and CDT were found in *C. difficile*. In 2020, based on the results of comprehensive laboratory studies, a diagnosis of “infectious anaerobic enterotoxemia of calves” was confirmed, with pathogenic bacteria *C. perfringens* and *C. difficile* toxintype B as the etiological agents. It was also established that the cause of cow mortality was acute intestinal infection caused by an association of *C. perfringens* and *C. difficile* type CDT pathogens [8, 9].

Russian and foreign scientists, as well as practical specialists, have proven that inadequate control over zoo-hygienic and zoo-sanitary conditions, lack of vaccination, and disinfection are some of the main reasons for outbreaks of clostridial infection in livestock enterprises (see

footnote 1) [4, 10, 11]. Worldwide, a wide range of clostridial antitoxins, bacterin-antitoxins, or whole-culture vaccines are used to protect animals from these diseases. For example, specific prophylaxis of *C. chauvoei* in cattle herds began in 1930 and continues to this day in areas with blackleg outbreaks [2]. This concept of annual planned vaccination of the population has yielded good results, leading to only occasional sporadic cases of the disease nowadays [12].

According to the Veterinary Department of the Sverdlovsk region, the number of animals vaccinated against blackleg is increasing every year. If in 2015, the proportion of vaccinated livestock was less than 10.0% of the total number of cattle in the region, by 2022, this figure had increased to 73.0% [see table 1].

Polyvalent vaccines against clostridiosis in cattle, sheep, and goats have been used since the 1950s [3, 12]. Until 2009, associated vaccines for the prevention of clostridial infections in cattle were not produced in Russia. The domestic market for veterinary immunobiological products included a vaccine against blackleg and a tetanus antitoxin<sup>3</sup>. The blackleg vaccine, developed by S.N. Muromtsev (1931) and modernized by F.I. Kagan and Ya.R. Kovalenko (1965), contained inactivated concentrated bacterial mass of *C. chauvoei*, adsorbed on aluminum oxide gel [3]. The tetanus antitoxin contains the *C. tetani* toxin inactivated by formalin, precipitated, and adsorbed on alum [4, 8]. Prophylaxis of malignant edema, necrotic hepatitis, and anaerobic enterotoxemia was not possible due to the absence of available drugs<sup>4</sup>. Currently, the State Register of Veterinary Medicines in the Russian Federation officially includes 15 vaccines against clostridiosis in agricultural animals, produced by ten domestic and five foreign manufacturers [see Table 2].

The immunogenicity and effectiveness of vaccines depend on many factors - the quantity and quality of the grown microorganisms, antigen, immunostimulants, adjuvant, preser-

<sup>3</sup>Popova A.S., Alexeeva I.G. Diagnosis of clostridiosis in cattle // Modern trends in the development of veterinary science and practice: Proceedings of the National (All-Russian) scientific-practical conference (Omsk, October 26, 2021). Omsk: Publishing House of Omsk State Agrarian University named after P.A. Stolypin, 2021, pp. 43–46.

<sup>4</sup>Kapustin A.V., Laishchev A.I., Sklyarov O.D., Abrosimova N.S. Development of a method to control the immunogenic activity of the associated vaccine against bovine clostridial clostridiosis // Russian Journal of Agricultural and Socio-Economic Sciences, 2017, vol. 63, N 3, pp. 170–175.



**Табл. 1.** Динамика вакцинации против ЭМКАР крупного рогатого скота, разводимого на территории Свердловской области

**Table 1.** Dynamics of vaccination against blackleg in cattle bred in the Sverdlovsk region

Year	Livestock population, heads	Vaccinated	
		heads	%
2015	232 734	23 207	9,9
2016	227 314	123 875	54,5
2017	225 678	112 996	50,1
2018	228 917	131 401	57,4
2019	218 837	139 002	63,5
2020	222 784	173 853	78,0
2021	211 353	180 960	85,6
2022	220 199	161 075	73,0

vatives, manufacturing technology, application scheme, and the protective properties of the animal's body [11, 13]. When vaccinating against dangerous clostridiosis, it's crucial to monitor the following criteria: verification of the drug dose, intensity of the macroorganism's immune response, duration of protective immunity after vaccination and revaccination [see footnote 2]. Since various types of clostridia are agents of different infectious diseases, it is believed that using associated and polyvalent vaccines is more effective for preventive measures in livestock farms [5, 10].

Currently, the most accessible clostridial vaccines on the Russian market are combined preparations against several clostridial species, often including antitoxins derived from several toxintypes of *C. perfringens*: toxintypes C and D are almost always included by manufacturers in clostridial vaccines, whereas toxintypes A and E are introduced in the compositions of combined preparations much less frequently. Additionally, vaccines usually contain antitoxins of several other clostridial species: *C. chauvoei*, *C. novyi*, *C. sordellii*, *C. septicum*, *C. tetani*. Researchers assert that systemic immunity developed to *C. perfringens* type A is sufficient to protect against associated intestinal diseases. Since *C. perfringens* is an intestinal pathogen, considering the local activity of its toxins, it is assumed that IgA

of mucous membranes plays a more important role than serum IgG in protecting against necro-hemorrhagic enteritis in cattle<sup>5</sup> [1, 14].

The high genetic variability of clostridia often makes some vaccines ineffective. Some of the produced vaccines do not contain frequently detected clostridial species, for example, *C. sordellii*, which causes gas gangrene in animals. Therefore, when developing a plan for anti-epizootic measures at livestock enterprises and choosing vaccines, comprehensive laboratory tests of animal biomaterial are necessary to determine the species and toxintype of clostridia [15].

Research on the development and improvement of vaccines against clostridiosis in livestock continues. Clinical trials of new drugs are being conducted, and results are published in open press to provide veterinarians with comprehensive information about scientific developments in this field. For example, A.V. Danilyuk et al. (see footnote 2) developed and introduced the first Russian vaccine against clostridiosis in cattle and sheep, which creates a strong immunity against *C. perfringens* types A, B, C, D, *C. chauvoei*, *C. tetani*, *C. novyi*, *C. septicum* for at least 12 months. A.V. Kapustin et al. [12] created a polyvalent inactivated vaccine against blackleg or emphysematous carbuncle (EMCAR) for cattle and sheep, which not only achieves a strong post-vaccination immunity in laboratory animals but also extends its duration from 7 to 12 months (compared to the 6-month immunity provided by the previously used vaccine). Furthermore, developers regularly publish data on the comparative analysis of the immunogenic activity of domestic and foreign vaccine samples, allowing veterinary specialists to choose the most effective drugs for their livestock enterprises [2, 13, 16].

The primary methodological approach in controlling diseases caused by *Clostridium* bacteria is prevention, consisting of three key components: 1) individual biosecurity programs against clostridiosis in each farm; 2) systematic and timely vaccination of the herd; 3) strict ad-

<sup>5</sup>Shkuratova I.A., Shilova E.N., Sokolova O.V. Veterinary and sanitary aspects of disease prevention of young cattle in modern industrial complexes // Veterinary Farm Animals, 2017, N 12, pp. 51–54.

**Табл. 2.** Иммунобиологические лекарственные средства против клостридиозов сельскохозяйственных животных

**Table 2.** Immunobiological drugs against clostridiosis of farm animals

No. n/a	Name of the medication, manufacturer	Components included in the vaccine
1	2	3
<i>Vaccines made from cultures of cells</i>		
1	Concentrated polyvalent hydro-aluminum oxide vaccine against bradsot, infectious enterotoxemia, malignant edema of sheep and dysentery of lambs (FSOE "Armavir Biofactory", Russia)	Formalin-inactivated vaccine strain cultures <i>C. septicum</i> , <i>C. oedematies</i> , <i>C. perfringens</i> types B, C, D
2	Inactivated vaccine against emphysematous carbuncle of cattle and sheep (FSOE "Armavir Biofactory", Russia)	Formalin-inactivated concentrated cultures of virulent strain <i>C. chauvoei</i> R-15
3	One Shot Ultra 8. Inactivated vaccine for prevention of clostridiosis and pasteurellosis in cattle (Zoetis, CIIIA)	1) formalin-inactivated bacteria cultures <i>Manheimia (Pasteurella) haemolytica</i> (strain NL 1009), 2) formalin-inactivated bacteria cultures <i>C. chauvoei</i> (strain F), <i>C. septicum</i> (strain A), <i>C. haemolyticum</i> (strain IRP135), <i>C. novyi</i> (strain 8296), <i>C. sordelli</i> (strain 5918), <i>C. perfringens</i> (type C, strain P8), C8), <i>C. perfringens</i> (type D, strain 317)
4	Blackleg. Vaccine against emphysematous carbuncle of cattle and sheep (FSOE "Stavropol Biofactory", Russia)	Formalin-inactivated strain cultures <i>C. chauvoei</i> R-15
5	Vaccine against animal clostridiosis. Polyvalent concentrated vaccine against bradsot, infectious enterotoxemia, malignant edema and animal dysentery (LLC "Agrovet", Russia)	Formalin-inactivated vaccine strain cultures <i>C. septicum</i> , <i>C. novyi</i> , <i>C. perfringens</i> types B, C, D,
6	Concentrated aluminum hydroxide formolvaccine against emphysematous carbuncle of cattle and sheep (FSOE "Armavir Biofactory", Russia)	Formalin-inactivated strain cultures <i>C. chauvoei</i> R-15
<i>Vaccines made from anatoxins and cell cultures</i>		
7	Coglavax. Polyvalent inactivated vaccine against clostridiosis in cattle and sheep (Ceva-Phylaxia Veterinary Biologicals Company, Hungary)	<i>C. perfringens</i> type A alpha-anatoxin, <i>C. perfringens</i> type C beta-anatoxin, <i>C. perfringens</i> type C epsilon-anatoxin D, <i>C. septicum</i> anatoxin, <i>C. novyi</i> anatoxin type B, <i>C. tetani</i> anatoxin, <i>C. chauvoei</i> anaculture
8	Equilis Prequenza Te. Vaccine for prevention of influenza and tetanus in horses (Intervet International, Netherlands)	Culture fluid of SPF cell culture of chicken embryos infected with recombinant canarypox virus (strain vCP2242) expressing the hemagglutinin gene of influenza virus A/equi-2/Ohio/03[H3N8] and strain vCP 1533 expressing the hemagglutinin gene of influenza virus A/equi-2/Newmarket/2/93[H3N8] and purified <i>C. tetani</i> anatoxin (30 IU).

Окончание табл. 2

1	2	3
9	Inactivated equine influenza and tetanus vaccine (FSOE "Kursk Biofactory", Russia)	Tetanus toxoid and culture fluid of a transvirginized MDCK cell line infected with equine influenza virus
10	Antox. Inactivated polyvalent anatoxin against sheep clostridiosis (FSOE "Stavropol Biofactory", Russia)	Mixture of toxins of <i>C. perfringens</i> strains types B (C) and D, <i>C. oedematiens</i> and bacterial cells of <i>C. septicum</i> strain inactivated with formalin
11	Klostbovac 8. Inactivated polyvalent vaccine against clostridiosis in sheep and cattle (Vetbiochemistry LLC, Russia)	Inactivated cultures of <i>C. chauvoei</i> and <i>C. septicum</i> strains, anatoxins of <i>C. novyi</i> (oedematiens) type B, <i>C. perfringens</i> types A, C, D, <i>C. tetani</i>
12	Toxipra plus. Inactivated vaccine against enterotoxemia, emphysematous carbuncle and tetanus (Laboratorios Hipra, Spain)	Beta and epsilon toxoids of <i>C. perfringens</i> types B, C and D, alpha toxoids of <i>C. novyi</i> type B, alpha toxoids of <i>C. septicum</i> , ana-cultures of <i>C. chauvoei</i> , toxoids of <i>C. tetani</i>
13	Pastanarm-8. Polyvalent inactivated associated vaccine against ruminant pasteurellosis and clostridiosis (FSOE Armavir Biofactory, Russia).	Mixture of formalin-inactivated anatoxin, <i>Clostridium</i> and <i>Mannheimia (Pasteurella) haemolytica</i> bacterial cultures
14	Antox-9. Inactivated vaccine against clostridiosis of farm animals (FSOE "Stavropol Biofactory", Russia)	Cell cultures of <i>C. chauvoei</i> , <i>C. septicum</i> , <i>C. novyi</i> (oedematiens) anatoxins types A and B, <i>C. perfringens</i> types A, C (B) and D, <i>C. sordellii</i> , <i>C. tetani</i> , inactivated with formalin
15	Cubolac. Polyvalent inactivated vaccine against clostridiosis in cattle and sheep (CZ Vaccines, Spain)	Cultures of <i>C. chauvoei</i> cells, alpha-, beta- and epsilon-anatoxins of <i>C. perfringens</i> types A, C, D, anatoxins of <i>C. septicum</i> , <i>C. novyi</i> type B, <i>C. sordellii</i> , inactivated with formaldehyde

herence to zoo-sanitary and zoo-hygienic rules at the agricultural enterprise [10].

Effective measures against clostridioses in livestock include vaccination. For example, preventive vaccination against EMCAR is conducted after calves reach the age of 3 months and up to 4 years. There are two types of vaccines - live (revaccination once a year) and inactivated (revaccination twice a year). In the event of a local epidemic in the cattle population, all animals are vaccinated as an emergency measure. Animals that have recovered retain strong immunity (calves up to 3 months, cows and bulls up to 4 years) [8].

For the prevention of malignant edema, a polyvalent antitoxin is used. When animals are infected with pathogenic *C. perfringens*, an-

tibacterial drugs are recommended. Premises where sick animals were kept are treated with disinfectants containing halogens. The disposal of dead animals is carried out in accordance with the requirements and rules of veterinary-sanitary supervision [9].

The main measures for preventing botulism in cattle include strict adherence to the rules of fodder preparation and storage, constant control over the zoo-sanitary condition of premises, feeding tables, and watering places. During outbreaks of botulism in farms, the use of antitoxin serum is recommended [4, 12]. During an outbreak, animal feeds of animal origin not subjected to heat treatment are excluded from the diet.

The basis for tetanus prevention is preventing injuries at agricultural enterprises. When animals are injured, first aid is provided with adherence to aseptic and antiseptic requirements. In cases of severe injuries (especially in the limbs), difficult births, and burn injuries to the skin, antitoxic antitetanus serum is administered no later than 12 hours after the injury to prevent tetanus. In farms where several cases of tetanus in animals are registered simultaneously, it is recommended to actively immunize the entire population with an antitoxin [3, 9].

The prevention of anaerobic enterotoxemia in calves involves feeding quality colostrum, proper feeding of pregnant cows, and immunization of adult cattle and calves (from 2-4 weeks of age) against clostridiosis, adhering to all veterinary-sanitary and zoo-hygienic rules for care and maintenance. Practicing veterinarians recommend administering antitoxic serum immediately after a calf's birth to create passive artificial immunity [2, 13, 15].

To combat dangerous clostridiosis, systematic disinfection of the premises, disposal of dead animals, and examination of feeds for the presence of spore forms of clostridia are also necessary [3]. One of the most important measures leading to the prevention of clostridiosis is specific prophylaxis. Vaccines and other alternative products can help minimize the need for antibiotics, preventing and controlling infectious diseases in the population, which is crucial for the future success of livestock farming (see footnote 5) [3, 5, 9].

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## ИНФОРМАЦИЯ ОБ АВТОРАХ

**Безбородова Н.А.**, кандидат ветеринарных наук, старший научный сотрудник

**Томских О.Г.**, кандидат ветеринарных наук, старший научный сотрудник

✉ **Кожуховская В.В.**, младший научный сотрудник; **адрес для переписки:** Россия, 620142, г. Екатеринбург, ул. Белинского, 112а; e-mail: Tetramegon@yandex.ru

**Порываева А.П.**, доктор биологических наук, ведущий научный сотрудник

**Сажаев И.М.**, младший научный сотрудник

## AUTHOR INFORMATION

**Natalya A. Bezborodova**, Candidate of Science in Veterinary Medicine, Senior Researcher

**Oksana G. Tomskikh**, Candidate of Science in Veterinary Medicine, Senior Researcher

✉ **Veronika V. Kozhukhovskaya**, Junior Researcher; **address:** 112a, Belinsky St., Yekaterinburg, 620142, Russia; e-mail: Tetramegon@yandex.ru

**Antonina P. Poryvaeva**, Doctor of Science in Biology, Lead Researcher

**Ivan M. Sazhaev**, Junior Researcher

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## РОЛЬ БИОЛОГИЧЕСКИ АКТИВНЫХ ДОБАВОК В СОЗДАНИИ ПОЛНОЦЕННЫХ ПИЩЕВЫХ ПРОДУКТОВ

✉ Углов В.А., Инербаева А.Т., Бородай Е.В.

*Сибирский федеральный научный центр агробиотехнологий Российской академии наук*

Новосибирская область, р.п. Краснообск, Россия

✉ e-mail: borodajelena@yandex.ru

Обобщены результаты анализа патентной, научно-технической информации и данные собственных исследований о перспективных биологически активных добавках: арабиногалактане, дигидрокверцетине, полисахаридах, биоактивированном зерне пшеницы. Сырьем для производства арабиногалактана и дигидрокверцетина служит преимущественно лиственница сибирская (*Lárix sibírica*) семейства сосновых Pinaceae. Разработаны эффективные экономичные технологии выделения их из древесины лиственницы. Приведены основные профилактические или лечебно-профилактические свойства арабиногалактана и дигидрокверцетина: увеличение иммунной активности организма человека, гепатопротекторные, гипополипидемические, антиоксидантные и другие свойства. Установлены функционально-технологические свойства этих добавок (высокая растворимость в воде, способность удерживать влагу и жир), позволяющие широко использовать их для производства разнообразных полноценных продуктов питания. Приведены основные санитарные правила, регламентирующие использование указанных добавок в пищевой промышленности. Представлены результаты анализа по использованию данных БАД в производстве разнообразных пищевых продуктов, обладающих комплексом лечебно-профилактических свойств. Обоснована роль дигидрокверцетина в сохранении качества пищевых готовых продуктов в течение длительного срока хранения. Полученный эффект тесно связан с его антиоксидантной активностью. Полисахариды (альгинат натрия, каррагинан и пектин свекловичный) используются как пищевые добавки, полученные из натурального сырья. Биоактивированное зерно пшеницы насыщает мясные продукты комплексом витаминов группы В, микро- и макроэлементами. Приведены основные результаты анализа патентной информации. В большинстве изобретений патентуются различные композиции с использованием биологически активных добавок, которые позволяют получать новые пищевые продукты с высокой пищевой, биологической ценностью, способные сохранять качественные показатели в течение длительного срока хранения.

**Ключевые слова:** арабиногалактан, дигидрокверцетин, пищевые добавки, полисахариды, биоактивированное зерно пшеницы

## THE ROLE OF BIOLOGICALLY ACTIVE ADDITIVES IN THE CREATION OF HEALTHY AND NUTRITIOUS FOOD PRODUCTS

✉ Uglov V.A., Inerbaeva A.T., Borodai E.V.

*Siberian Federal Scientific Centre of Agro-BioTechnologies of the Russian Academy of Sciences*

Krasnoobsk, Novosibirsk region, Russia

✉ e-mail: borodajelena@yandex.ru

The results of the analysis of patent, scientific and technical information and the own research data on promising biologically active additives: arabinogalactan, dihydroquercetin, polysaccharides, bioactivated wheat grain are summarized. The raw material for the production of arabinogalactan and dihydroquercetin is mainly Siberian larch (*Lárix sibírica*) of the pine family Pinaceae. Effective eco-

nomical technologies of their extraction from larch wood have been developed. The main prophylactic or therapeutic properties of arabinogalactan and dihydroquercetin are given: increase in the immune activity of the human body, hepatoprotective, hypolipidemic, antioxidant and other properties. Functional and technological properties of these additives (high solubility in water, ability to retain moisture and fat) have been established, allowing their wide use for the production of a variety of nutritious and healthy food products. The basic sanitary rules regulating the use of these additives in the food industry are given. The results of the analysis on the use of these dietary supplements in the production of various food products with a complex of therapeutic and preventive properties are presented. The role of dihydroquercetin in preserving the quality of food prepared products during a long shelf life is substantiated. The resulting effect is closely related to its antioxidant activity. Polysaccharides (sodium alginate, carrageenan and beetroot pectin) are used as food additives derived from natural raw materials. Bioactivated wheat grain saturates meat products with a complex of B vitamins, micro- and macroelements. The main results of the patent information analysis are presented. In most inventions various compositions using biologically active additives are patented, which allow to obtain new food products with high nutritional, biological value, capable of maintaining quality parameters during a long shelf life.

**Keywords:** arabinogalactan, dihydroquercetin, food additives, polysaccharides, bioactivated wheat grain

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**Conflict of interest**

The authors declare no conflict of interest.

The production of modern, ecologically safe food products that align with the concept of balanced nutrition is a key factor in maintaining and improving the health of the population in the Russian Federation. Excessive, unbalanced, and poor-quality nutrition leads to excessive strain on the body's organs and systems, depletes the endocrine-metabolic apparatus, which manifests clinically in civilization diseases. According to numerous researchers, improving this situation may be associated with the use of new biologically active supplements (BAS), whose widespread implementation could enhance the human body's resistance to adverse environmental factors, improve the immune status, and increase resistance to the most common diseases (cardiovascular system, oncology, diabetes). Unfortunately, in recent years, due to various social and economic factors, the psycho-emotional state of people has been deteriorating.

The purpose of this work is to assess the role of modern biologically active supplements in creating complete food products based on the analysis of patent and scientific-technical information.

The study examined biologically active supplements such as arabinogalactan, dihydroquercetin, and polysaccharides – sodium alginate, carrageenan, beet pectin, as well as bioactivated wheat grain.

All analytical, physicochemical, and technological research was carried out in the Department of Food Systems and Biotechnology of the SFSCA RAS (formerly the Department of Quality Problems of SibNITIP) using modern methods and instrumental equipment. Siberian larch (*Larix sibirica*) of the pine family Pinaceae is the raw material for the production of arabinogalactan and dihydroquercetin. All food additives from natural polysaccharides of plant



origin – food-grade sodium alginate produced by the Arkhangelsk Experimental Seaweed Plant from brown algae, carrageenan MP 414 produced by Frutarom Ltd. (Israel) from red algae, and beet pectin produced in the Kabardino-Balkarian Republic of the Russian Federation – are approved by the State Sanitary and Epidemiological Supervision of the Ministry of Health of the Russian Federation. Soft and hard wheat varieties of 1–4 classes according to GOST 52554–2006, regionalized in the Novosibirsk region, were used as raw material for obtaining sprouted grains.

Comparative-analytical research methods were used. Patent research and search for scientific-technical information were carried out using the websites of FIPS, Espacenet, WIPO, PUBMED, and Google.

Analysis of patent and scientific-technical information has revealed that researchers and manufacturers are primarily focused on biologically active supplements (BAS) with a broad spectrum of effects. In recent years, plant polysaccharides are increasingly being used as therapeutic and prophylactic agents. Arabinogalactan, derived from Siberian larch wood (family Pinaceae), exhibits high biological activity, including immunomodulating, hepatoprotective, and hypolipidemic effects [1, 2]. A significant advantage of arabinogalactan is its prebiotic properties. Its high biological activity, virtually non-existent toxicity, and functional-technological properties (good solubility in cold water, ability to retain moisture, bind fat) open up wide possibilities for its use. Arabinogalactan's application is regulated by SanPin 2.3.2.1078–01, classifying it as a thickener, gelling agent, and stabilizer. Methodical recommendations of the State Sanitary-Epidemiological Standardization of the Russian Federation No. 2.3.1.1915-04 (2004) establish adequate and maximum permissible consumption levels of arabinogalactan at 10 to 20 g per day.

Bioflavonoids also belong to the group of BAS with high biological activity. Recently, there has been increasing interest in dihydro-

quercetin. It reduces the risks of developing cardiovascular and oncological diseases and thrombosis, enhances the immune activity of the body, stimulates the central nervous system [3, 4]. Its use in food production significantly prolongs shelf life, but its most significant advantage is its high antioxidant activity. This is largely due to the regeneration of natural or synthetic antioxidants by transferring a hydrogen atom from the phenolic hydroxyls of the dihydroquercetin molecule [5].

Affordable, economically justified technologies for obtaining arabinogalactan and dihydroquercetin, as well as practically inexhaustible raw material resources, may contribute to their widespread implementation in the production of biologically complete food products. The use of dihydroquercetin in production is regulated by SanPiN 2.3.2.1078–01, where it is classified as an antioxidant. The Technical Regulation of the Customs Union “Safety Requirements for Food Additives, Flavorings, and Technological Aids” (TR TS 029/2012) allows the use of dihydroquercetin as an antioxidant in food production (Appendix 2), and GOST 33504–2015 presents the main requirements for it.

The analysis of scientific and technical information allows concluding that these two biologically active additives have a wide range of applications in the production of food products with high biological value. Currently, dihydroquercetin is used in more than 100 types of food products and medicinal preparations.

V.G. Shelepov et. al. developed a biologically active biopolymeric matrix based on chitosan succinate (patent RU 2698455), which includes arabinogalactan, chitosan, and dihydroquercetin. This matrix is characterized by its high complexing ability, bioavailability, and hydrophilicity with respect to various water-insoluble compounds and chemical elements. It also has enhanced penetration capability, exhibiting a range of therapeutic and prophylactic properties – antioxidant, radioprotective, and antiseptic. The authors improved the technology for producing meat pate from venison, offal, veg-

etable raw materials using the biopolymer matrix, which allows for the production of finished products with therapeutic and prophylactic properties. It was established that this technology extends the shelf life of finished products due to the use of bionanocomposite in the recipe, highlighting its economic feasibility [6].

In recent years, the proportion of meat with disrupted traditional autolysis processes has increased, complicating its use in the production of high-quality meat products. Some researchers have studied the effect of arabinogalactan on the quality of sausage products made from stress-sensitive chicken meat. The water-binding capacity of experimental minced meat samples using arabinogalactan was 67%, compared to 62% in the control group, and heat treatment losses in the control samples were 34%, compared to 28% in the experimental ones. Arabinogalactan in meat products weakens oxidation processes during production and storage (acid number is 14.7–25.0 lower, peroxide number 14.3–23.0% lower). Studies on the use of these two additives in dairy, confectionery, bakery, and other industries are also known [7].

Research aimed at preserving the quality of food products for extended storage periods is also relevant. The positive effect is associated with dihydroquercetin as an antioxidant, which helps to inhibit the process of peroxide oxidation, not only extending shelf life but also increasing the biological value of the products.

Valuable are the innovative developments focused on increasing the solubility of BAS for the production of semi-finished products with predetermined properties based on supra-molecular complexes of these substances with water-soluble polymers, particularly polysaccharides and chitosan. The research results of these BAS have been patented. For example, the semi-smoked venison sausage (RU 2294115) includes the BAS Anabarin, known for its pronounced immunostimulating and adaptogenic effect. Its use helps to overcome states of fatigue and psycho-emotional stress, protects human internal organs and systems from stress dam-

age, and normalizes cholesterol levels in the blood. A notable effect of Anabarin is its ability to enhance mental and physical performance, especially in unfavorable climatic conditions (northern and Arctic basin territories). Thus, the issue of more effective and comprehensive use of meat raw materials is resolved, simultaneously obtaining high-quality food products with prophylactic properties.

A multifunctional composition (patent RU 2603896) intended for therapeutic, prophylactic, and dietary nutrition, containing the biologically active additive Flavocen (dihydroquercetin), is distinguished by its increased content of vitamins, essential substances, macro- and microelements. It can maintain the nutritional value over a long storage period, while reducing the cost of raw materials. A composition with ascorbic acid and dihydroquercetin (patent RU 2437546) has been developed for producing a cream-protein product. This invention allows for the production of a product with probiotic properties, high nutritional and biological value, excellent sensory indicators, and an extended shelf life.

As a result of incorporating dihydroquercetin in the product recipe, a method for producing concentrated sterilized milk for functional nutrition (patent RU 2425575) has been developed, which has enhanced biological value and improved organoleptic characteristics.

Arabinogalactan and dihydroquercetin are also used in the confectionery industry. For example, the method for producing a mass for sugar confectionery products on a fat basis (patent RU 2211576) increases the quality of sugar confectionery products, enhances nutritional value, extends shelf life, and gives the products prophylactic properties. A food composition for producing crispy rye-wheat bread (patent RU 2500109) allows for an optimal recipe for this food product, suitable for consumption by a wide group of people, including those with obesity and diabetes.

Polysaccharides are among the most important food additives. Including alginates, carra-

geenans, and pectins in the recipes of various therapeutic and prophylactic food products, in addition to improving sensory properties, helps enhance food safety due to their complexing properties. Studies have been conducted on developing methods for extracting alginates from *Fucus* seaweed, flax seeds, and using them in the production of bakery products or fortifying wheat flour [8–11]. There is interest in research on the impact of pectin polysaccharides or their mixture with food acids on the quality of confectionery products [12, 13].

Researchers from the Department of Food Systems and Biotechnology of the Siberian Federal Research Centre of Agro-BioTechnologies (formerly SibNIITIP) have developed a recipe for a minced meat semi-finished product from broiler chicken meat with sodium alginate, carrageenan, and sugar beet pectin. Ecological assessments of plant additives were conducted on white Wistar rats at the State Research Center of Virology and Biotechnology “Vector” of the Russian Consumer Rights Protection and Human Well-Being Surveillance Service. Optimal dosages of polysaccharides for producing ecological food products from poultry meat were determined. Technical specifications and technical instructions for the poultry meat semi-finished product have been developed. Patents for the following inventions have been obtained: No. 2336719 “Method of removing cadmium and lead from animal organism”, No. 2391876 “Method of obtaining semi-finished products from meat of farm animals and poultry”, No. 2375913 “Method of feeding animals and birds”, No. 2612781 “Method of manufacturing a combined meat product”, No. 2644958 “Method of manufacturing a meat product of functional purpose”.

For producing bioactivated grain, soft and hard wheat of 1–4 grades, intended for food purposes and meeting the quality and sanitary-hygienic indicators of GOST 52554-2006, were used. Bioactivation of wheat grain was carried out by soaking at room temperature for 22 hours in a 1:0.5 ratio of grain to drinking water. The

purpose of grain activation is to synthesize and activate inactive enzymes, which, under the influence of soaking, achieve the development of all reserve substances in the grain. The bioactivated wheat was crushed using an impact cutting mill designed by SibNIITIP. The milling method is patented by the institute. Based on the research, a method for making semi-smoked sausage with bioactivated wheat grain was developed (patent RU № 2547715). As a result of the introduction of bioactivated wheat grain in the product formulation in the amount of 10 wt.% it is possible to increase the content of B vitamins, fiber, which are deficient in human nutrition and contribute to detoxification of the human body and reduction of cholesterol. The obtained result is achieved as a result of intensive bioactivation processes occurring during moisture saturation of grain. According to some authors, the amount of vitamin E in germinating wheat grains increases 3 times compared to dry grain. Also, the amount of micro- and macroelements increases: potassium from 350 to 850 mg/100 g, calcium from 45 to 70, phosphorus from 423 to 1100, magnesium from 146 to 400, iron from 3.9 to 10 mg/100 g. Wheat grain after germination also becomes an excellent source of vitamin B complex (B1 increases from 0.46 to 2 mg/100 g, B2 - from 0.23 to 0.7, B6 - from 0.5 to 3, folic acid from 0.04 to 0.35 mg/100 g) [14]. Vitamins of this group improve the nervous system, eliminate dysbacteriosis, increase the body's resistance to infectious diseases and rheumatism. This is a new full-fledged product with a health-improving effect.

In general, there is growing interest in plant polysaccharides with high biological activity, non-toxicity, and non-allergenic properties, and the raw material potential for their production in Russia is quite significant. New biologically active additives with a wide range of actions are emerging in the food and processing industries, capable of competing with traditional pharmacopoeial drugs and Western counterparts.

The broader use of polysaccharides such as arabinogalactan, dihydroquercetin, sodium

alginate, carrageenan, sugar beet pectin, and bioactivated wheat grain in the production of wholesome food products will significantly expand the range of products with prophylactic and therapeutic-prophylactic properties, which could greatly improve the health of the Russian population.

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## ИНФОРМАЦИЯ ОБ АВТОРАХ

✉ **Углов В.А.**, кандидат биологических наук, старший научный сотрудник; e-mail: Naukoved1939@yandex.ru

**Инербаева А.Т.**, кандидат технических наук, ведущий научный сотрудник; e-mail: atinerbaeva@yandex.ru

**Бородай Е.В.**, старший научный сотрудник, **адрес для переписки:** Россия, 630501, Новосибирская область, р.п. Краснообск, а/я 463; e-mail: borodajelena@yandex.ru

## AUTHOR INFORMATION

✉ **Vladimir A. Uglov**, Candidate of Science in Biology, Senior Researcher; e-mail: Naukoved1939@yandex.ru

**Aigul T. Inerbaeva**, Candidate of Science in Engineering, Lead Researcher; e-mail: atinerbaeva@yandex.ru

**Elena V. Borodai**, Senior Researcher; **address:** PO Box 463, Krasnoobsk, Novosibirsk Region, 630501, Russia; e-mail: borodajelena@yandex.ru

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**CONCLUSION**

**REFERENCES.** The number of sources must be at least 15. The list of references includes only peer-reviewed sources: articles from scientific journals and monographs. Self-citation of no more than 10% of the total number. The bibliography list should be designed as a general list in the order of mention in the text, it is desirable to refer to sources 2-3 years old. The rules for the list of references are in accordance with GOST R 7.05-2008 (requirements and rules for compiling a bibliographical reference). In the text the reference to the source is marked by a serial number in square brackets, for example [1]. Literature in the list is given in the languages in which it was published. In the bibliographic description of the publication, it is necessary to include all authors, without abbreviating them by one, three, etc. It is unacceptable to abbreviate the names of articles, journals, publishing houses.



If it is necessary to refer to abstracts, dissertations, collections of articles, textbooks, recommendations, manuals, GOSTs, information from websites, statistical reports, articles in socio-political newspapers, etc., such information should be placed in a *footnote* at the end of the page. Footnotes are numbered in Arabic numerals, placed page by page through numbering.

**Attention!** Theoretical, review and problem articles can have any structure, but must contain an abstract, keywords, list of references.

## EXAMPLE OF REFERENCES in Russian and English and FOOTNOTES

### REFERENCES (in Russian):

#### **Monograph**

*Klimova E.V.* Field crops of Zabaikalya: monograph. Chita: Poisk, 2001. 392 p.

#### **Part of a book**

*Kholmov V.G.* Minimum tillage of coulisse-strip fallow for spring wheat with intensification of arable agriculture in southern forest-steppe of Western Siberia // Resource-saving tillage systems. Moscow: Agropromizdat, 1990. pp. 230-235.

#### **Periodical publication**

*Pakul A.L., Lapshinov N.A., Bozhanova G.V., Pakul V.N.* Technological grain qualities of spring common wheat depending on the system of soil tillage // Siberian Herald of Agricultural Science. 2018. vol. 48. № 4. pp. 27-35. DOI: 10.26898/0370-8799-2018-4-4.

### REFERENCES (in English):

References are compiled in the same order as the Russian version, according to the following rules:

Names and surnames of the authors are given in the established way of transliteration, English title of the article, *transliteration of the name of the Russian-language source (for example through the site: <https://antropophob.ru/translit-bst>) = English title of the source*. The order of presentation for a monograph is the following: city, English name of the publisher, year, number of pages; for a journal: year, number, pages). (In Russian).

**Example:** Author A.A., Author B.B., Author C.C. Title of article.

Transliteration of the authors. English title of the article.

*Zaglavie jurnala = Title of Journal*, 2012, vol. 10, no. 2, pp. 49–54.

*Transliteration of the source = English name of the source*

#### **Monograph**

*Klimova E.V.* *Field crops of Zabaikalya*. Chita, Poisk Publ., 2001, 392 p. (In Russian).

#### **Part of a book**

*Kholmov V.G.* Minimum tillage of coulisse-strip fallow for spring wheat with intensification of arable agriculture in southern forest-steppe of Western Siberia. *Resource-saving tillage systems*, Moscow, Agropromizdat Publ., 1990, pp. 230–235. (In Russian).

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*Pakul A.L., Lapshinov N.A., Bozhanova G.V., Pakul V.N.* Technological grain qualities of spring common wheat depending on the system of soil tillage. *Sibirskii vestnik sel'skokhozyaistvennoi nauki = Siberian Herald of Agricultural Science*, 2018, vol. 48, no. 4, pp. 27–35. (In Russian). DOI: 10.26898/0370-8799-2018-4-4.

### FOOTNOTES:

Quoted text<sub>1</sub>.

*1Klimova E.V., Andreeva O.T., Temnikova G.P.* Ways to stabilize food production in Transbaikalia // Problems and prospects of perfecting zonal farming systems in modern conditions: materials of the scientific and practical conf. (Chita, October 16-17 2008). Chita, 2009, pp.36-39.

**Digital Object Identifier – DOI** (when the cited material has it) should be indicated at the end of the bibliographic reference.

Example:

*Chu T., Starek M.J., Brewer M.J., Murray S.C., Pruter L.S.* Assessing lodging severity over an experimental maize (*Zea mays* L.) field using UAS images // Remote Sensing, 2017. Vol. 9. P. 923. DOI: 10.3390/rs9090923.

The DOI of the article should be checked on the website <http://search.crossref.org/> or <https://www.citethisforme.com>. To do this, enter the title of the article in English in the search bar.

## FIGURES, TABLES, SCREENSHOTS AND PHOTOGRAPHS

The figures must be of good quality, suitable for printing. All figures must have captions. The caption must be translated into English. Figures should be numbered in Arabic numerals according to the order in the text. If there is only one figure in the text, it is not numbered. References to figures should be formatted as follows: “Fig. 3 indicates that ...” or “It is indicated that ... (see Fig. 3)”. The caption under the figure includes a figure number and its title. “Figure 2. Description of vital processes.” The translation of the figure caption should be placed after the figure caption in Russian.

Tables should be of good quality, suitable for printing. Tables suitable for editing are preferred, not scanned or as figures. All tables should have headings. The title of the table should be translated into English. Tables should be numbered in Arabic numerals according to the order in the text. If there is only one table in the text, it is not numbered.

References to tables should be formatted as follows: “Table 3 states that ...” or “It is stated that ... (see Table 3)”. The title of the table includes a table number and its title: “Table 2. Description of Vital Processes.” The translation of the table title should be placed after the table title in Russian.

Photos, screenshots and other non-drawn illustrations must be uploaded separately as \*.jpeg files (\*.doc and \*.docx if the image has additional marks). The resolution of the image should be >300 dpi. The image files should be given a name corresponding to the figure number in the text. In the description of the file a caption should be given separately, which should correspond to the name of the picture placed in the text.

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