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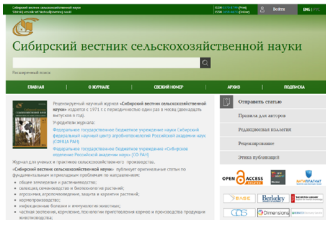

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Классификация и картографирование сельскохозяйственных культур с использованием дистанционного зондирования и машинного обучения

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Представлены результаты исследований по классификации и картографированию сельскохозяйственных культур с помощью машинного обучения (МО) с использованием данных дистанционного зондирования. Исследования проведены в 2022, 2023 гг. на территории землепользования хозяйств, расположенных в Новосибирской области. Использованы изображения, полученные с Sentinel-2 и Landsat 8-9. Признаками для обучения моделей МО выступали шесть спектральных полос и пять вегетационных индексов за разные даты вегетационных периодов. Применяли алгоритмы МО с контролируемым обучением: XGBoost, KNN, RF и SVM, а также нейронную сеть deep-FNN. Модели XGBoost, KNN и RF показали высокую точность классификации – 93–97% при разрешении 30 м/пиксель и 80–90% при разрешении 90 м/пиксель. Модель deep-FNN показала наименьшие результаты с точностью от 78 до 92% при разрешении 30 м/пиксель. Общее снижение точности на 8–12% при разрешении 90 м/пиксель в сравнении с разрешением 30 м/пиксель подчеркивает важность масштаба для эффективного распознавания культур. Также обучение моделей на объединенных данных спутников Sentinel-2 и Landsat 8-9 при разрешении 30 м/пиксель дало более высокие значения метрики F1-score, чем на данных отдельно по каждому из этих спутников. Различные метрики оценки (F1-score и ROC-AUC-score) подтвердили, что модель XGBoost была наиболее производительной и точной. Лучшая общая классификация достигнута для кукурузы, ячменя, однолетних и многолетних трав, а также залежи, с некоторым снижением точности для овса, гороха, вики и пшеницы мягкой озимой. Наименьшая точность отмечена при классификации картофеля, ярового рапса, пшеницы мягкой яровой и пара. Результаты исследований подчеркивают значимость выбора модели МО и масштаба разрешения спутниковых снимков для успешной классификации сельскохозяйственных культур.

Ключевые слова: классификация сельскохозяйственных культур, дистанционное зондирование, машинное обучение, картографирование

Crop classification and mapping using remote sensing and machine learning

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Research results on crop classification and mapping using machine learning (ML) with remotely sensed data are presented. Studies were conducted in 2022, 2023 on the land use of the farms located in the Novosibirsk region. Sentinel-2 and Landsat 8-9 images were used. The features for training

the ML models were six spectral bands and five vegetation indices for different dates of the growing seasons. XGBoost, KNN, RF and SVM supervised learning ML algorithms and deep-FNN neural network were applied. The XGBoost, KNN and RF models showed high classification accuracies of 93–97% at 30 m/pixel resolution and 80-90% at 90 m/pixel resolution. The deep-FNN model showed the lowest results with an accuracy of 78 to 92% at 30 m/pixel resolution. The overall 8–12% reduction in accuracy at 90 m/pixel resolution compared to 30 m/pixel resolution emphasizes the importance of scale for effective crop recognition. Also, training the models on the combined Sentinel-2 and Landsat 8-9 satellite data at 30 m/pixel resolution yielded higher values of the F1-score metric than on the data separately for each of these satellites. Various evaluation metrics (F1-score and ROC-AUC-score) confirmed that the XGBoost model was the best performing and most accurate. The best overall classification was achieved for corn, barley, annual and perennial grasses, and fallow, with some decrease in accuracy for oats, peas, vetch and soft winter wheat. The lowest accuracy was observed in the classification of potatoes, spring rape, soft spring wheat and fallow. The results of the research emphasize the importance of ML model selection and satellite imagery resolution scale for successful crop classification.

Keywords: crop classification, remote sensing, machine learning, mapping

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INTRODUCTION

Spatial data in the form of large-scale crop maps at the level of a specific land use, municipal district or region are necessary for the development and management of government subsidy allocation and crop market planning. In addition, the growing demand for crop insurance has increased the practical requirements for crop mapping. Crop identification using traditional methods such as field surveys, although accurate, has limitations. Mapping a single crop plot takes considerable time and access to these plots can be difficult. Field surveys therefore remain an expensive approach to crop mapping, and regulatory agencies may not have the manpower to carry out such work on the ground. To meet this need, there is a need for online crop statistics at the plot level, including for tracking declared and actual crops sown. This can be achieved using remote sensing technologies and artificial intelligence methods. [1–3].

Since the early 1970s, classification of vegetation using remote sensing data has been used as an alternative to traditional field studies. However, it has not yet been possible to completely eliminate field surveys. They are necessary for quantitative assessment of the structure of agricultural crops, preparation of data and classification algorithms, and validation of models [4, 5]. Over the past two decades, the importance of research in crop mapping using remote sensing has continued to grow [6, 7]. The widespread popularity of satellite imagery over other platforms is due to its resolution and open access. Research has shown that multispectral remote sensing images obtained with appropriate sensors are suitable for crop mapping [8–11]. The second most widely used crop mapping technology is synthetic aperture radar (SAR) [12, 13]. The use of hyperspectral images remains uncommon, as does the use of terrestrial lidar scanner. For crop classification, a single sensor is usually used, as pre-processing

the images from multiple sensors can be challenging. In global practice, images from the Sentinel and Landsat series of satellites are currently the most commonly used in this subject area [3, 14].

Machine learning (ML) and deep learning (DL) models have shown the ability to classify crops from medium and high-resolution satellite images with high accuracy[1] [15]. The algorithms and models used for crop mapping are varied and grouped into three categories:

– parametric ML classifiers (e.g. logistic regressions, hidden Markov models);

– nonparametric ML classifiers (e.g. support vector machine, decision tree, random forest, k-nearest neighbors, etc.);

– DL classifiers (neural networks of various modifications).

Parametric machine learning classifiers work by making certain assumptions about the statistical distribution of the data.

Nonparametric ML classifiers make no assumptions about the distribution of the data. The mathematical basis of DL allows neural networks to learn to recognize patterns in the data that is fed to them during the training process [16–18].

A review of the effectiveness of crop classification models using ML and DL based on remote sensing data conducted in the paper [19] showed that the most successful were random forest (RF), support vector machine (SVM), extreme gradient boosting (XGBoost) and neural networks (CNN, LSTM). These models achieved the highest overall accuracy in numerous studies (386 publications). However, the range of variation in the overall accuracy indicator was quite large. This indicates that the choice of the classifier and specific features significantly affects the accuracy of the crop mapping process [20, 21]. A possible solution to the problem of accuracy in crop classification may be to combine images from different satellites, which helps to reduce the time gap in the data. This technology is called multimodal remote sensing, and the classification efficiency of combined images from data [22] is superior to that of individual images.

The purpose of the study was to assess the accuracy of classification of agricultural crops by various models of ML and DL using remote sensing data and to carry out their mapping on the territory of the studied land use objects.

MATERIAL AND METHODS

Research on the classification and mapping of agricultural crops using remote sensing data was carried out in 2022 and 2023 on the land use territory of the EEP "Praktik" of the Novosibirsk State Agrarian University and the ES "Elitnaya" of the Siberian Federal Scientific Centre of Agro-BioTechnologies of the Russian Academy of Sciences, located in the Novosibirsk district of the Novosibirsk region (54°54'20.15"N, 82°52'20.06"E and 54°54'57"N, 82°57'6"E), with a total area of 950 ha. Classification and mapping were carried out for crops cultivated in farms: potatoes, soft spring wheat, soft winter wheat, corn, annual grasses, spring rape, barley, vetch, oats, peas, perennial grasses. The structure of land use of farms also includes complete fallow and unused lands (layland).

The work used images obtained from Sentinel-2 (Operational Land Imager (OLI)) and Landsat 8-9 (Thermal Infrared (TIRS) Collection 2), available for free download from Sentinelhub (https://apps.sentinel-hub. com) and the US Geological Survey (https:// earthexplorer.usgs.gov), respectively. Images with cloudiness below 75%, obtained from June 1 to August 31 were used. All satellite data were matched in accordance with atmospheric and radiometric correction. Scanned pictures of the images were converted to vector form and divided into objects in the form of a regular grid of 30 × 30 m (7657 data on the EEP "Praktik" object and 23,191 on the ES "Elitnaya" object) and 90 × 90 m (887 data on the EEP "Praktik"

[1] *Wu F., Maleki R., Oubara A., Gómez D., Eftekhari A., Yang G.* Machine Learning Approaches for Crop Identification from Remote Sensing Imagery: A Review // International Conference on Soft Computing and Pattern Recognition. Cham: Springer Nature Switzerland, 2023, pp. 325–336. DOI: 10.1007/978-3-031-27524-1_31.

object and 3081 on the ES "Elitnaya" object). Data containing information on spectral characteristics and vegetation indices were calculated using the "zonal statistics" function in Quantum GIS (QGIS).

Spectral bands from both sensor systems were used: blue, green, red, near infrared (NIR), shortwave infrared SWIR1 and SWIR2, as well as five vegetation indices: NDVI (Normalized Difference Vegetation Index), LAI (Leaf Area Index), NDMI (Normalized Difference Moisture Index), NDWI (Normalized Difference Water Index), GNDVI (Green Normalized Difference Vegetation Index).

A preliminary field survey was conducted to collect reliable data on crops. The boundaries of the plots were recorded digitally using SW maps in shp-file format and integrated into QGIS. Satellite images were also loaded into QGIS, their characteristics were developed, unnecessary stripes were eliminated, and vegetation indices were calculated. The values of the spectral characteristics of pixels from multispectral satellite images and vegetation indices were referenced in a single coordinate system 2505 Pulkovo 1942/Gauss-Kruger CM 87E to construct a data set. The first set consisted of the data from the Sentinel-2 satellite, the second – from Landsat 8-9 data, the third included a combination of the data from these satellites.

The input data for ML and DL were georeferenced contours of agricultural plots with crops placed on them, formed as classes of spatial objects using the "regular grid" function in QGIS. The classes were characterized by attribute characteristics of spectral bands and vegetation indices (11 features in total). Preliminary processing included data normalization, handling of missing values, and dividing the data into training and test sets in a ratio of 80 and 20%, respectively. The test sample was formed using the method of complete randomization. The correlation analysis was also conducted to identify a high correlation between the features and the recognizable classes. No high correlation was found, so all 11 features were included in the ML and DL models. In 2022, 12 crops (classes) were cultivated on the territory of the studied farms, of which two classes were present in both land use objects. In 2023, 11 crops (classes) were cultivated, of which two classes were also present in both objects. The distribution of the data by classes was unbalanced.

To build crop classification models, ML algorithms with supervised learning were used: Support Vector Machine – SVM, K-Nearest Neighbor – KNN, Random Forest – RF and XGBoost. ML algorithms were trained using a dataset containing pre-classified crop samples. Besides, to classify the data the deep feed-forward neural network – deep-FNN – with three open layers was used. This algorithm is designed to autonomously learn and extract hierarchical representations of data using interconnected layers of nodes. The deep-FNN neural network was trained over 20 epochs. Standard Python libraries for ML were used to build and train the models (numpy, pandas, skikit-learn, matplotlib, etc.).

The selection of metrics for assessing classification accuracy was carried out taking into account a specific model. For the task of identification by SVM, KNN, RF and XGBoost algorithms with an unbalanced distribution of data by classes, the weighted avg F1-score was used, since this metric is a harmonic mean between precision and recall and allows taking into account not only the proportion of the correctly classified positive examples, but also the ability of the model to detect all positive examples, as well as the ROC-AUC-score metric. Categorical accuracy was used as an error metric to evaluate the performance of deep-FNN. To evaluate each class separately, the classification_report method from the scikit-learn library was used, which provides information for each class separately, as well as the average weighted F1-score values[2].

The models were validated by field recording of the placement of agricultural crops through-

[2]Dealing with unbalanced data. https://habr.com/ru/companies/otus/articles/769242/

out the entire territory of land use objects and comparing the results obtained with the classification results. Based on the results of the XGBoost model for the classification of agricultural crops and its validation, crop mapping was carried out in the QGIS software package.

RESULTS AND DISCUSSION

Crop mapping based on remote sensing remains challenging due to the high local variability and temporal dynamics of agricultural crops. These properties result in high intra-class variance of the spectral-temporal signal, which increases with the size and diversity of the study area, the number of crops to be recognized, and the variability of meteorological conditions. To address the problems associated with spectral-temporal variations, data with sufficient spatial resolution and high temporal density of observations are usually required, which can be combined in the used ML approaches with verified field data.

The peculiarity of our research was that optical remote sensing data with a resolution of 10 and 20 m per pixel of the Sentinel-2 satellite and 30 m per pixel of Landsat 8-9, expressed in a raster model, were transformed into a vector model using QGIS functions.

Using a vector model of data representation and grid construction, the spectral characteristics of pixels and the values of vegetation indices are combined in a single geodatabase of features in the form of tables, thereby creating the ability to work with ML algorithms and optimizing computing resources. To reduce classification errors due to the use of the images taken on the same day, the maximum possible number of images available for download during crop vegetation was used. In total, there were 26 such images for both satellites, years and land use objects.

At the first stage of the research, the accuracy of the ML algorithms was tested with all data sets in terms of years and land use objects (see Table 1). Almost all ML models showed fairly high performance and accuracy of crop classification when using the weighted average F1-score as a unit of measurement, but some features should be noted. Thus, the XGBoost model demonstrated the best results on all data sets in both years of research, while the classification accuracy was 94–97% at a scale of 30 m/pixel and 80–90% at a scale of 90 m/pixel.

The KNN model demonstrated approximately the same classification accuracy as the XGBoost model. A slightly lower classification accuracy was observed when implementing the RF model – 93–96% at a scale of 30 m/pixel and 80–88% at a scale of 90 m/pixel. The lowest classification accuracy rates were obtained when implementing the deep-FNN model, which varied within 78–92% at a scale of 30 m/pixel and within 56–79% at a scale of 90 m/pixel. The obtained results can be explained by the fact that ML algorithms can process tabular data types, while deep neural networks (DNNs) have difficulties processing such data. According to the authors of articles on machine learning on specialized Internet resources Habr (https://habr.com/ru/articles) and Kaggle (https://www.kaggle.com/), DNNs showed good results when working with images and text, but when processing tabular data, DNNs were inferior in classification accuracy to ML algorithms. The use of DL for analyzing tabular data remains understudied, and variants of ensemble ML models still dominate in most applications[3–5]. In this regard, classical ensemble approaches (XGBoost and RF) demonstrated higher classification accuracy than DNNs.

[3]Jaykumaran. Training 3D U-Net for Brain Tumor Segmentation (BraTS2023-GLI) Challenge. AVAILABLE AT: https://learnopencv.com/3d-u-net-brats. (accessed 24.10.2024).

[4]Learning from tabular data. AVAILABLE AT: https://habr.com/ru/articles/534186/ (accessed 29.10.2024).

[5]Data Science Trends on Kaggle! AVAILABLE AT: https://www.kaggle.com/code/shivamb/data-science-trends-on-kaggle (accessed 29.10.2024).

The F1-score metric values when training models on various Sentinel-2 and Landsat 8-9 satellite data at different resolutions, years of research and land use objects have unstable characteristics. This can be explained by the uneven number of observation days and the number of predictors formed depending on this number. In addition, the accuracy of the classification depended not only on the number of predictors, but also on the distribution of survey days over

Табл. 1. Результаты оценки точности моделей машинного обучения по метрике F1-score
Table 1. Results of assessing the accuracy of machine learning models using the F1-score metric

Model	EEP "Praktik"			ES "Elitnaya"		
	Sentinel-2	Landsat 8-9	Landsat + Sentinel	Sentinel-2	Landsat 8-9	Landsat + Sentinel
2022						
Scale 30 m/pixel						
KNN	0,95	0,92	0,95	0,96	0,96	0,96
SVM	0,92	0,87	0,91	0,94	0,93	0,95
RF	0,95	0,93	0,95	0,95	0,96	0,96
XGBoost	0,95	0,94	0,95	0,96	0,97	0,97
deep-FNN*	0,92	0,85	0,92	0,88	0,78	0,88
Scale 90 m/pixel						
KNN	0,81	0,82	0,77	0,86	0,83	0,85
SVM	0,76	0,75	0,71	0,82	0,77	0,82
RF	0,80	0,80	0,80	0,83	0,84	0,84
XGBoost	0,81	0,80	0,81	0,84	0,86	0,86
deep-FNN*	0,69	0,72	0,71	0,62	0,56	0,64
2023						
Scale 30 m/pixel						
KNN	0,97	0,96	0,97	0,96	0,94	0,96
SVM	0,96	0,91	0,96	0,94	0,86	0,94
RF	0,96	0,96	0,96	0,96	0,95	0,96
XGBoost	0,97	0,97	0,97	0,97	0,96	0,97
deep-FNN*	0,91	0,91	0,92	0,87	0,85	0,89
Scale 90 m/pixel						
KNN	0,89	0,85	0,89	0,85	0,80	0,86
SVM	0,81	0,74	0,81	0,85	0,72	0,82
RF	0,88	0,85	0,88	0,87	0,83	0,86
XGBoost	0,90	0,87	0,90	0,89	0,85	0,88
deep-FNN*	0,69	0,74	0,78	0,74	0,76	0,79

*For deep-FNN, the categorical_accuracy metric was used.

the vegetation period, namely: the more evenly the survey days are distributed in time, the higher the accuracy of the classification.

However, it can be argued that higher classification accuracy for all ML models on both land use objects and in both years of research was obtained at a scale of 30 m/pixel compared to a scale of 90 m/pixel. Also, training of all models on combined data from Sentinel-2 and Landsat 8-9 satellites at a scale of 30 m/pixel showed more stable values of the F1-score metric. The results of the deep neural network with the FNN architecture (deep-FNN) were not included in the further analysis due to its low classification ability.

In ML, there are many metrics for assessing the accuracy and performance of models, each with its own advantages and disadvantages. However, a single universal metric that could optimally assess the quality of model performance in all cases has not yet been developed. In this regard, to assess the performance of models, it is not enough to use only one metric, but it is necessary to compare the values of two or more metrics that are most suitable for a given task.

The weighted average F1-score does not always allow for an unambiguous comparison of the performance of models due to its dependence on the threshold probability value. In this regard, in addition to the F1-score, this study uses the ROC-AUC-score metric, which allows for an adequate comparison of the performance of different models with an unbalanced data set (the higher the value of this metric, the higher the classification ability of the models). Thus, the highest values of the ROC-AUC-score metric were shown by the XGBoost method for both years of research for both objects using the combined data of the Sentinel-2 and Landsat 8-9 satellites at a scale of 30 m/pixel. In 2022, for the ES "Elitnaya" object, this indicator was 0.978, for the EEP "Praktik" object – 0.923, in 2023 – 0.972 and 0.979, respectively. The KNN method also showed close values of the ROC-AUC-score metric. In 2022, for the EEP "Praktik" object, it was only 0.1% less than the XGBoost method, in 2023 – 0.7%. At a scale of 90 m/pixel, the maximum ROC-AUC-score value was also shown by the XGBoost method. Thus, in 2023, for the EEP "Praktik" object, this metric is equal to 0.877, for the ES "Elitnaya" object – 0.895, in 2022, for the EEP "Praktik" object, the ROC-AUC-score is equal to 0.714, for the ES "Elitnaya" object – 0.896.

To visualize the accuracy assessment of ML models using ROC-AUC-score, a graph showing the results of crop classification when analyzing combined data (Sentinel-2 and Landsat 8-9) at a resolution of 30 m/pixel and 90 m/pixel for 2023 is presented as an example (see Fig. 1).

The ROC curve displays the ratio between the proportion of classes from the total number of feature carriers correctly classified as carrying the feature and the proportion of classes from the total number of classes not carrying the feature, incorrectly classified as carrying the feature. Typically, the ROC curve has a parabolic shape with varying curvature, and the higher the curvature, the better the model works. Fig. 1 shows that the ROC curve for the analysis of images at a scale of 30 m/pixel for the XGBoost method has better characteristics than all other ML methods for both the EEP "Praktik" object and the ES "Elitnaya" object. The worst ROC curve indicators are noted for the SVM method, the KNN and RF methods occupy an intermediate position. The same patterns were observed for the ROC curve when analyzing images at a scale of 90 m/pixel, but with reduced curvature. Taking into account the analysis of the model accuracy assessment by two metrics (F1-score and ROC-AUC-score), it can be concluded that the most productive and accurate at different scale values for both years of research and for both land use objects was the extreme gradient boosting method (XGBoost), the second place was taken by the k-nearest neighbors method (KNN), the third place was occupied by the random forest (RF). The lowest accuracy rates were shown by the support vector machine (SVM), i.e. it can be concluded that this algorithm is

poorly suited for solving problems of this type. At the same time, the combined data from Sentinel-2 and Landsat 8-9 satellites at a scale of 30 m / pixel had a greater informative capacity for training models in crop classification problems.

The overall crop classification accuracy was determined using the output of the XGBoost model on the fused 30 m/pixel satellite data averaged over 2022, 2023 (see Table 2).

The highest overall accuracy for land use objects was obtained when classifying corn, barley, annual grasses, as well as perennial grasses and layland. The recognition accuracy of the above crops was 0.2–2.0% higher than the weighted average F1-score. For oats, peas, soft winter wheat and vetch, the overall classification accuracy was 0.5–2.0% lower than the weighted average F1-score. The lowest overall accuracy was obtained when classifying potatoes, spring rape, soft spring wheat and fallow.

Based on the results of classification of remote sensing images using the XGBoost model, land use maps of the EEP "Praktik" and the ES "Elitnaya" were created, containing 11 identified classes of agricultural crops, as well as layland and fallow for 2022 (a) and 2023 (b) (see Fig. 2).

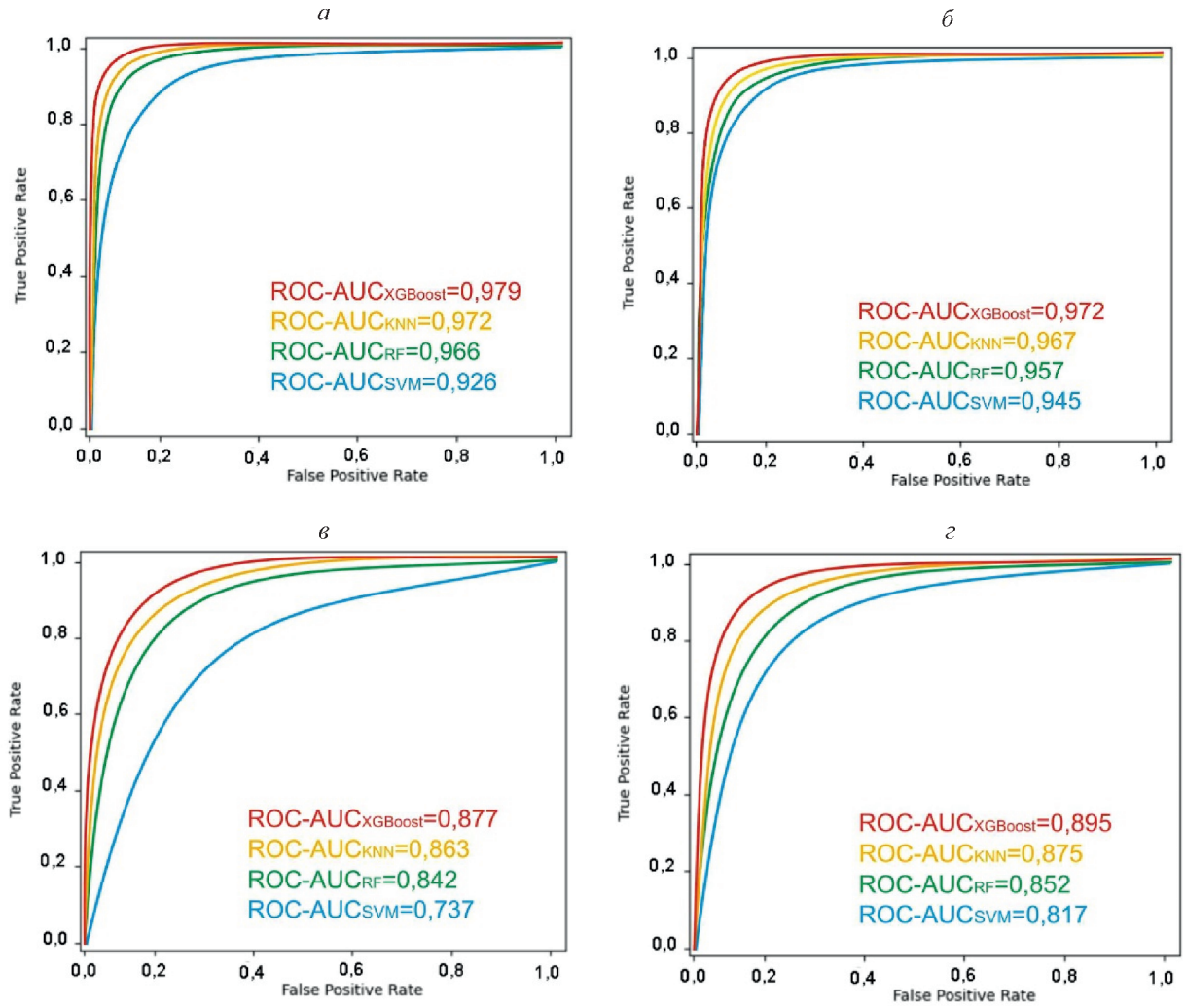

Рис. 1. ROC-кривые для разных методов машинного обучения. Масштаб 30 м/пиксель: *а* – УОХ «Практик»; *б* – ОС «Элитная»; масштаб 90 м/пиксель: *в* – УОХ «Практик», *г* – ОС «Элитная»

Fig. 1. ROC curves for different machine learning methods. Scale 30 m/pixel: *а* – EEP "Praktik", *б* – ES "Elitnaya"; scale 90 m/pixel: *в* – EEP "Praktik", *г* – ES "Elitnaya"

Mapping of agricultural crop species in the study areas was carried out using geoinformation technologies based on their classification (identification) using data analysis obtained from satellite images. ML models allow for efficient processing of large volumes of vector information from space images and contribute to increased classification accuracy. This opens up new opportunities for researchers and agronomists, allowing them to identify crop species and make informed agricultural decisions based on up-to-date information on their spatial localization.

Табл. 2. Общая точность классификации сельскохозяйственных культур моделью XGBoost

Table 2. Overall accuracy of crop classification by the XGBoost model

Class	Culture	Accuracy, %
1	Potato	83,5
2	Soft spring wheat	91,0
3	Layland	97,5
4	Soft winter wheat	95,0
5	Perennial grasses	96,7
6	Spring rape	88,0
7	Fallow	92,5
8	Corn	98,5
9	Annual grasses	98,0
10	Barley	98,5
11	Vetch	96,0
12	Oat	94,5
13	Peas	94,5
Weighted average F1-score		96,5

CONCLUSIONS

1. Evaluation of the ML models using the weighted average F1-score showed fairly high performance and accuracy of crop classification by the XGBoost, KNN, and RF algorithms, with the exception of the SVM method. The best results on all data sets in both years of research were demonstrated by the XGBoost model with a classification accuracy of 94 to 97% at a scale of 30 m/pixel and 80–90% at a scale of 90 m/pixel. The KNN model demonstrated approximately the same classification accuracy as the XGBoost model. A slightly lower classification accuracy was observed when implementing the RF model – 93–96% at a scale of 30 m/pixel and 80–88% at a scale of 90 m/pixel. The lowest classification accuracy rates were obtained when implementing the deep-FNN model, which varied within 78–92% at a scale of 30 m/pixel, and within 56–79% at a scale of 90 m/pixel.

2. The accuracy of crop classification on similar datasets was 8–12% lower at 90 m/pixel than at 30 m/pixel. This highlights the significant impact of scale on crop recognition accuracy. Also, training all models on combined Sentinel-2 and Landsat 8–9 data at 30 m/pixel showed higher F1-score values than on individual data from each of these satellites.

3. The analysis of the model accuracy assessment by two metrics (F1-score and ROC-AUC-score) showed that the most productive and accurate at different scale values for both years of research and for both land use objects was the extreme gradient boosting method (XGBoost), slightly inferior to it in accuracy were the k-nearest neighbors (KNN) and random forest (RF) methods. The lowest accuracy rates were shown by the support vector machine (SVM). The combined data from Sentinel-2 and

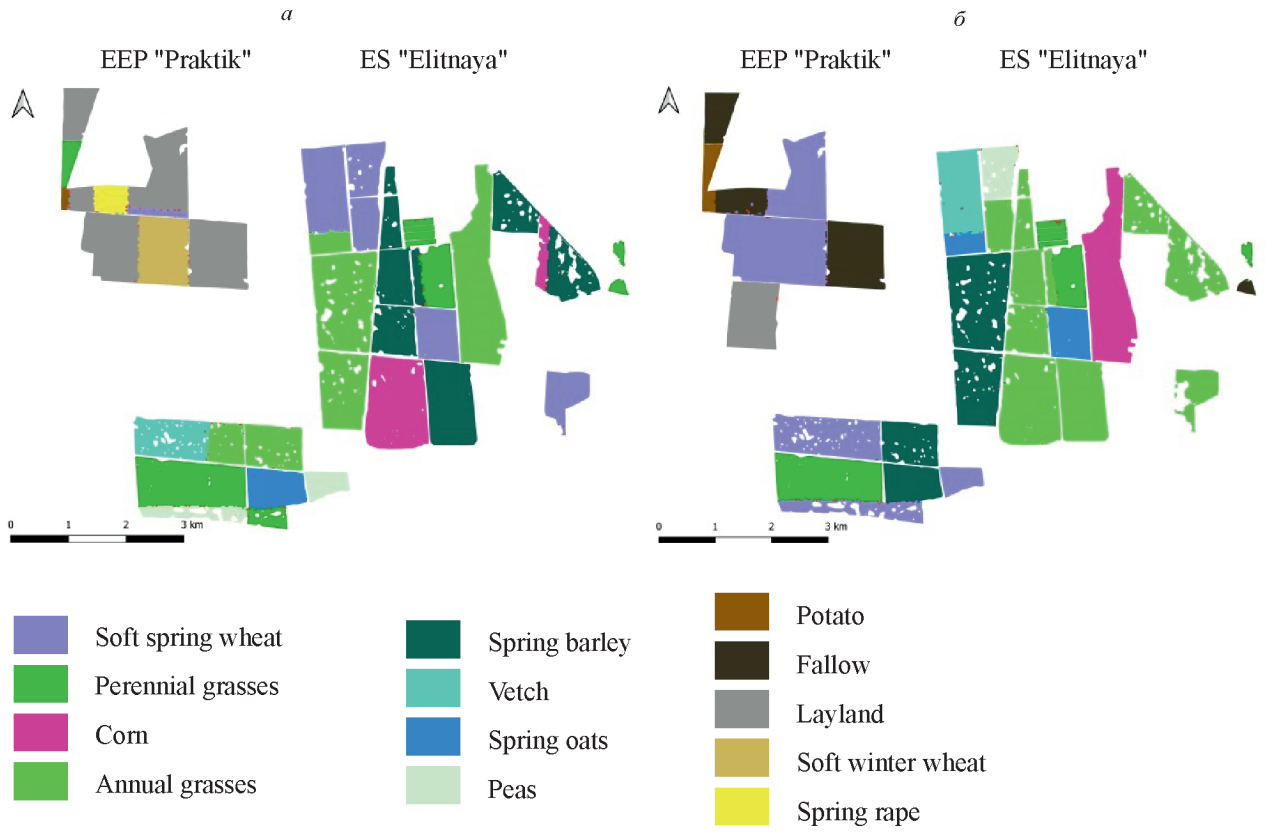

Рис. 2. Карты классификации сельскохозяйственных культур УОХ «Практик» и ОС «Элитная»
Fig. 2. Maps of classification of agricultural crops of the EEP "Praktik" and ES "Elitnaya"

Landsat 8-9 satellites at a scale of 30 m/pixel had a greater informative capacity for training models.

4. The highest overall accuracy (average for 2022, 2023) for land use objects was obtained for corn, barley, annual grasses, as well as for perennial grasses and layland when classifying the combined Sentinel-2 and Landsat 8-9 data at a resolution of 30 m/pixel using the XGBoost model. The recognition accuracy of the above crops was 0.2–2.0% higher than the weighted average F1-score. For oats, peas, soft winter wheat and vetch, the overall classification accuracy was 0.5–2.0% lower than the weighted average F1-score. The lowest overall accuracy was obtained when classifying potatoes, spring rape, soft spring wheat and fallow.

5. The use of remote sensing and ML allows the creation of crop placement maps in land use space, thereby reducing the labor costs of field geolocation and the creation of digital online statistics of cultivated crops. These maps can also be used to monitor crops and forecast yields.

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Сила роста как важный фактор жизнеспособности семян сои

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Высокая жизнеспособность семян представляет собой одно из важнейших условий получения богатых урожаев различных сельскохозяйственных культур. При этом сила роста является значимым фактором жизнеспособности семян, оказывает влияние на урожайность, позволяет прогнозировать качество урожая, тем самым снижая затраты на минеральные удобрения и препараты-биостимуляторы. В статье приведены результаты испытаний четырех сортов сои селекции Всероссийского научно-исследовательского института орошаемого земледелия: нового перспективного сорта ВНИИОЗ 4, а также районированных сортов ВНИИОЗ 86, ВНИИОЗ 31 и Волгоградка 2. Цель исследования заключалась в сравнительной оценке жизнеспособности семян перспективного и районированных сортов сои на основе изучения показателей силы роста семян (отношение числа пробившихся ростков к числу высеянных всхожих семян; масса надземной и корневой частей растений в пересчете на 100 ростков). Закладку опыта и последующий учет результатов проводили в лабораторных условиях по утвержденной методике определения силы роста семян. Установлено, что за годы проведения испытаний (2021–2023) наибольшей силой роста обладали семена сорта ВНИИОЗ 4. Среди изучаемых сортов у данного сорта зафиксирована наибольшая величина зеленой массы и массы корневой части растений (109,6 и 47,9 г соответственно). Новый сорт показал максимальный средний процент нормальных ростков, вышедших на поверхность (92,7%), одновременно с минимальной долей непроросших семян (1,3%) и ростков с признаками болезни (0,7%). Также отмечено отсутствие непроросших семян в 2022 и 2023 гг.

Ключевые слова: соя, селекция, сорт, сила роста, жизнеспособность семян

The power of growth as an important factor in the viability of soybean seeds

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High seed viability is one of the most important conditions for obtaining high yields of soybeans. At the same time, vigor is a significant factor of seed viability; it influences yield, allows predicting the quality of yield, thereby reducing the cost of mineral fertilizers and biostimulant preparations. The article presents the results of the tests of four varieties of soybean breeding of the All-Russian Research Institute of Irrigated Agriculture: a new promising variety VNIIOZ 4, as well as the released varieties VNIIOZ 86, VNIIOZ 31 and Volgogradka 2. The purpose of the study was to comparatively assess the viability of seeds of the promising and released soybean varieties on the basis of studying the indicators of seed vigor (the ratio of the number of germinated sprouts to the number of sown germinated seeds; the mass of the aboveground and root parts of plants in terms of 100 sprouts). Experimental design and subsequent recording of the results were carried out in laboratory conditions according to the

approved methodology for determining the seed vigor. It was found that during the test years (2021–2023) the seeds of the variety VNIIOZ 4 had the highest growth vigor. Among the studied varieties, this variety recorded the highest value of green mass and root mass of the plants (109.6 and 47.9 g, respectively). The new variety showed maximum average percentage of normal shoots emerged on the surface (92.7%), along with minimum percentage of ungerminated seeds (1.3%) and shoots showing signs of disease (0.7%). The absence of ungerminated seeds was also observed in 2022 and 2023.

Keywords: soybean, selection, variety, growth vigor, seed viability

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INTRODUCTION

Increasing the status of soybeans as the most important agricultural crop, which serves as the main raw material for food industry enterprises, is one of the priority directions of agricultural production and contributes to solving the problem of food security on the scale of not only a specific region, but also the country as a whole [1–5]. The increase in production of such a crop as soybean, which is responsive to growing conditions, must necessarily be accompanied by appropriate scientific support, primarily in the field of selection and seed production [6–8]. The guarantee of obtaining stable yields of high-quality products along with the use of highly adaptive varieties is the use of high-quality seed material [9, 10].

High seed viability is one of the most important conditions for obtaining rich harvests of any agricultural crop, including soybeans. Factors influencing viability (field and laboratory germination, growth energy) have been studied in sufficient detail[1] [11, 12]. At the same time, such an indicator as germinative power, which represents the ability of seeds to break through to the surface of the soil (or other substrate) and is a complex indicator of their biological properties, has undeservedly fallen by the wayside.

This indicator is of great importance, as it determines the ability of seeds to form healthy plants, affects crop yields, allows predicting the quality of the crop, reducing the costs of mineral fertilizers and biostimulant preparations [13, 14].

The purpose of the study is a comparative assessment of the viability of seeds of the promising and released soybean varieties bred by the All-Russian Research Institute of Irrigated Agriculture (VNIIOZ) based on a study of germinative power rate indicators (the ratio of the number of germinated sprouts to the number of sown germinated seeds; the mass of the aboveground and root parts of the plants per 100 sprouts).

[1]*Mysak E.V., Selikhova O.A., Tikhonchuk P.V.* Influence of photoperiod on sowing qualities of seeds and main elements of soybean productivity // Achievements of science and technology of AIC, 2014, N 6, pp. 51–53.

MATERIAL AND METHODS

The experiment was conducted by VNIIOZ employees in 2021–2023. The objects of the study were zoned varieties VNIIOZ 86, VNIIOZ 31, Volgogradka 2 and a new promising variety VNIIOZ 4. The experiment was laid out and the results were recorded in laboratory conditions according to the method for determining the germinative power[2].

The number of seeds sown – 50 pcs. The experiment was repeated three times. The seeds were sown at a depth of 0.1 m in glass vessels with sand moistened to 60% of the total moisture capacity and covered with a 7 cm layer of air-dry sand with a grain size of 1.0–2.5 mm. Germination was carried out in the light at a temperature of 16–18 °C. The germinative power was determined on the 10th day. On the day of the count, the shoots that emerged on the surface were cut flush with the sand surface, then counted and weighed. Separate records were kept of germinated, ungerminated, swollen and rotten seeds of each of the studied varieties.

RESULTS AND DISCUSSION

As part of the study, the following main indicators of the germinative power were taken into account: the number of germinated sprouts as a percentage of the sown germinated seeds, as well as the mass of the aboveground and root parts of the plants per 100 sprouts.

According to the obtained results, among the four varieties, the maximum average value of green mass and root system mass was recorded for the promising variety VNIIOZ 4 (109.6 and 47.9 g, respectively). At the same time, the highest indicators for this variety were noted in 2023 – 131.6 and 66.2 g, respectively (see Table 1).

The number of normal sprouts in the VNIIOZ 86 variety was maximum in 2023 (90%), minimum – in 2021 (78%). On average, during the study, the share of the sprouts that did not break through to the surface was 11.3%, undergerminated seeds – 3.3%. No sprouts with signs of disease or curved were noted (see Table 2).

In the VNIIOZ 31 variety, the highest number of normal sprouts was recorded in 2022 (88%), the lowest – in 2021 (84%). The share of undergerminated seeds ranged from 4 (2022 and 2023) to 10% (2021). The average percentage of sprouts that did not emerge on the surface was 8%. No sprouts with signs of disease or curved ones were found (see Table 2).

The Volgogradka 2 variety had the highest number of normal sprouts in 2023 (90%) and the lowest in 2021 (80%). The share of undergerminated seeds averaged 4% over three years. The average percentage of sprouts with signs of disease and curved sprouts was 0.7%, and those that did not break through to the surface were 9.3% (see Table 2).

The new promising variety VNIIOZ 4 had the highest number of normal sprouts in 2023 (96%), the lowest in 2021 (90%). The average percentage of the sprouts that did not emerge on the surface was 5.3%, the sprouts with signs of disease and curved – 0.7%. The share of undergerminated seeds averaged 1.3% (see Table 2).

Табл. 1. Масса надземной и корневой частей растений в пересчете на 100 ростков, г
Table 1. Weight of the above-ground and root parts of plants per 100 sprouts, g

Variety	Herbage				Root weight			
	2021	2022	2023	Average for 3 years	2021	2022	2023	Average for 3 years
VNIIOZ 4	103,2	94,0	131,6	109,6	41,4	36,2	66,2	47,9
VNIIOZ 86	95,6	59,8	93,0	82,8	45,6	44,2	41,8	43,9
VNIIOZ 31	82,0	101,0	86,4	89,8	42,8	47,6	46,8	45,7
Volgogradka 2	87,4	107,0	113,8	102,7	34,0	47,6	50,8	44,1

[2]GOST 12040–66. Seeds of agricultural crops. Method for determining growth strength // Seeds and planting material of agricultural crops: collection of standards. Moscow: Publishing House of Standards, 1973, 408 p.

Табл. 2. Показатели силы роста семян изучаемых сортов, шт./%
Table 2. Indicators of seed growth vigor of the studied varieties, pcs./%

Variety	Sprouts			Not germinated seeds
	normal	not germinated	with signs of disease and crooked	
		2021		
VNIIOZ 4	45/90	2/4	1/2	2/4
VNIIOZ 86	39/78	7/14	0/0	4/8
VNIIOZ 31	42/84	3/6	0/0	5/10
Volgogradka 2	40/80	8/16	0/0	2/4
		2022		
VNIIOZ 4	46/92	4/8	0/0	0/0
VNIIOZ 86	44/88	6/12	0/0	0/0
VNIIOZ 31	44/88	4/8	0/0	2/4
Volgogradka 2	44/88	2/4	0/0	2/8
		2023		
VNIIOZ 4	48/96	2/4	0/0	0/0
VNIIOZ 86	45/90	4/8	0/0	1/2
VNIIOZ 31	43/86	5/10	0/0	2/4
Volgogradka 2	45/90	4/8	1/2	0/0
		Average for 3 years		
VNIIOZ 4	44,0/92,7	2,3/5,3	0,3/0,7	0,7/1,3
VNIIOZ 86	42,6/85,3	2,8/11,3	0/0	0,8/3,3
VNIIOZ 31	43,0/86,0	4,0/8,0	0/0	3,0/6,0
Volgogradka 2	43,0/86,0	2,3/9,3	0,3/0,7	1,3/4,0

CONCLUSION

Thus, it was established that over the years of the experiment, the seeds of the new promising variety VNIIOZ 4 had the greatest germinative power. With an average number of normal sprouts of 92.7%, there were no undergerminated seeds in 2022 and 2023 (an average of 1.3% over three years) and a minimum proportion of the sprouts with signs of disease (0.7%). In addition, this variety was distinguished by maximum indicators of green mass (131.6 g) and root mass (66.2 g). Based on the results of the study, the VNIIOZ 4 variety was sent to undergo the state variety testing procedure.

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Молекулярно-генетический полиморфизм *Glycine soja* Sieb. & Zucc. из популяции Дальневосточного региона

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Glycine soja Sieb. & Zucc. является предком культурной сои и имеет более широкую генетическую изменчивость по многим хозяйственно ценным признакам, в том числе по содержанию белка. В настоящее время наиболее популярным инструментом генетического анализа принято считать молекулярные маркеры, которые активно используются в изучении генетического разнообразия. Цели исследования – дифференциация форм дикой сои из коллекции Федерального научного центра «Всероссийский научно-исследовательский институт сои», а также составление на основе SSR-анализа молекулярно-генетических формул, необходимых для дальнейшей селекционной работы. Объектом исследования послужили 47 форм дикой сои из разных районов Амурской области и 4 формы из ближайших ареалов (Хабаровский и Приморский края, Китай). Для изучения молекулярно-генетического полиморфизма проведен стандартный ПЦР-анализ на готовой реакционной смеси с использованием 20 пар SSR-праймеров. Впервые на основе оценки степени полиморфизма ДНК дикой сои по микросателлитам составлены генетические формулы исследуемых коллекционных генотипов, построена дендрограмма для выявления их генетического разнообразия, установлена степень генетического родства. Максимальное количество уникальных аллелей (по девять) обнаружено у форм дикой сои, произрастающих в Благовещенском районе Амурской области (КБл-91) и Хабаровском крае (Хаб-1). Незначительным генетическим разнообразием отличались образцы из Архаринского района, в которых отмечено не более трех уникальных аллелей для каждой формы. Так как в селекционных программах рекомендуется использовать формы дикой сои с наибольшим разнообразием уникальных аллелей, апробированная маркерная система может быть применена для генетического дифференцирования форм дикой сои.

Ключевые слова: *Glycine soja*, генетическое разнообразие, ДНК-маркеры, микросателлитные локусы, SSR-анализ, молекулярно-генетические формулы

Molecular genetic polymorphism of *Glycine soja* Sieb. & Zucc. from the population of the Far East region

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Glycine soja Sieb. & Zucc. is the ancestor of cultivated soybean and has a wider genetic variability in many economically valuable traits, including protein content. Currently, the most popular tool of genetic analysis is considered to be molecular markers, which are actively used in the study of genetic diversity. The objectives of the study are differentiation of wild soybean forms from the collection of the Federal Research Center "All-Russian Scientific Research Institute of Soybean", as well as compilation of molecular-genetic formulas necessary for further breeding work on the basis of SSR-analysis. The object of the study were 47 forms of wild soybean from different districts of the Amur region and

4 forms from the nearest habitats (Khabarovsk and Primorsky Territories, China). A standard PCR analysis was performed on the finished reaction mixture using 20 pairs of SSR primers. For the first time, based on the assessment of the degree of polymorphism of wild soybean DNA by microsatellites, genetic formulas of the studied collection genotypes were compiled, a dendrogram was constructed to identify their genetic diversity and the degree of genetic kinship was established. The maximum number (9 each) of unique alleles was detected among the *G. soja* forms of the Blagoveshchensk region (KBl-91) and Khabarovsk region (Hub-1). The samples from the Arkharinsky district were characterized by insignificant genetic diversity, in which no more than three unique alleles for each form were noted. As it is recommended to use forms of *G. soja* with the greatest variety of unique alleles in breeding programs, the marker system tested in the work can be used for the genetic differentiation of *G. soja*.

Keywords: *Glycine soja*, genetic diversity, DNA markers, microsatellite loci, SSR-analysis, molecular genetic formulas

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INTRODUCTION

Genetic diversity is the most important condition for increasing the yield. At various periods, cultivated soybean (*Glycine max* (L.) Merr.) has undergone significant genetic changes, including the introduction of local varieties to other regions of the country and the world. Active use of selective breeding has led to a decrease in genetic diversity, causing a number of problems in the cultivation of this crop [1]. At the same time, wild relatives of soybeans, on the contrary, have a relatively high level of genetic diversity. In this regard, among the wild forms of soybeans, Ussuri soybean (*Glycine soja* Sieb. & Zucc.) can be especially distinguished, which, compared to *G. max*, has an abundance of alleles and can be used to improve varieties. *G. soja* is able to hybridize with cultivated soybeans due to the absence of a selection barrier between them, which allows it to be used to increase genetic diversity and expand the gene pool[1].

Analysis of literature sources showed that wild soybeans are phylogenetically diversified and adapted to different habitats, have good resistance to biotic and abiotic stresses (low temperature, dehydration, salt stress, high levels of phenylpropanoids and isoflavones)[2] [2, 3]. Therefore, *G. soja* can be a source of genes for increasing the resistance of *G. max*[3] [1, 3]. Hy-

[1]*Chung G., Singh R.J.* Broadening the genetic base of soybean: a multidisciplinary approach // Critical Reviews in Plant Sciences, 2008, vol. 27, N 5, pp. 295–341.

[2]*Robison J., Arora N., Yamasaki Yu., Saito M., Boone J., Blacklock B., Randall S.* Glycine max and Glycine soja are capable of cold acclimation // Journal of Agronomy and Crop Science, 2017, vol. 203, N 6, pp. 553–561.

[3]*Li Y.H., Li W., Zhang Ch., Yang L., Chang R.-Zh., Gaut B., Qiu L.-J.* Genetic diversity in domesticated soybean (Glycine max) and its wild progenitor (Glycine soja) for simple sequence repeat and single-nucleotide polymorphism loci // New Phytologist, 2010, vol. 188, N 1, pp. 242–253.

bridization of *G. max* and *G. soja* contributes to the creation of new cultivars with increased stress resistance [4].

Collection of various genetic resources and selection of valuable traits through botanical classification, external morphological characteristics and genetic evaluation are of great importance[4]. At this stage, molecular markers are considered the most popular tool for genetic analysis [5], especially SSR markers [6, 7]. They are not subject to environmental influences and are not limited in quantity, can be effectively used in the management of genetic resources, for the analysis of the diversity of quantitative traits, and for improving selection tools [8]. The use of SSR analysis to study wild soybeans will allow for genetic monitoring of these forms and will contribute to the preservation of genetic material in gene banks [9].

There are currently global gene banks that work to conserve wild soybean germplasm. For example, China has the largest collection of wild soybean germplasm in Southeast Asia, numbering 6,172 samples. The Chung G. collection (South Korea) contains 6,012 samples. The gene bank of the National Institute of Agricultural Sciences in Japan contains 1,131 samples [10, 11].

The Amur Region is home to the northern distribution area of wild soybeans, the genetic potential of which has been poorly studied. In this regard, the objectives of our study were to differentiate the forms of wild soybeans from the collection of the Federal Research Center "All-Russian Scientific Research Institute of Soybean" (FRC ARSRIS), as well as to compile molecular genetic formulas based on SSR analysis, which are necessary for further breeding work.

MATERIAL AND METHODS

The object of the study was 51 forms of wild soybean from the collection of the Federal Research Center "All-Russian Scientific Research Institute of Soybean". The forms were selected in six districts of the Amur Region (Arkharinsky - KA, Belogorsky - KBel, Blagoveshchensky - KBl, Zeisky - KZ, Mikhailovsky - KM, Tambovsky - KT), in Primorsky (No. 5683) and Khabarovsk Territories (Khab-1), as well as in the territory of the People's Republic of China (No. 5673, 5674) (see Fig. 1). In 2023, collection samples were grown on the basis of the selected forms in the experimental field of the Federal Research Center "All-Russian Scientific Research Institute of Soybean" (Sadovoye village, Tambovsky District, Amur Region) (see Fig. 2).

Ten seeds were selected from each sample for molecular genetic analysis. Total DNA was extracted using the DNA-Extran reagent kit (RPC “Sintol”, Russia) from pre-prepared soybean seeds (0.05 g). The concentration and quality of double-stranded DNA were measured using an EzDrop 1000 nano-spectrophotometer (BlueRay Biotech, Taiwan). The isolated DNA was diluted to 100 ng/µl.

PCR analysis was performed in triplicate using the above SSR primers for which the annealing temperatures were optimized. The final reaction volume was 25 µl and consisted of:

а) 12.5 µl of the ready-to-use reaction mixture "BioMaster HS-Taq PCR-Color (2×)" (OOO "Biolabmix", Russia), containing 100 mM Tris-HCl with pH = 8.5 (at 25 °C), 100 mM KCl, 0.4 mM of each deoxynucleoside triphosphate, 4 mM MgCl2, 0.06 units of activity/µl Taq DNA polymerase, 0.2% Tween-20, HS-Taq DNA polymerase stabilizers and dyes;

б) 10 ng of the extracted DNA sample;

в) 10 pM of forward and reverse primers;

г) 9.5 µl sterile water.

Amplification of the isolated soybean DNA fragments was carried out using a Real-time CFX96 amplifier (Bio-Rad Laboratories Inc., USA) in the following temperature-time regime: initial denaturation at 95 °C for 5 min; then 35 amplification cycles, including denaturation at 95 °C for 10 s, primer annealing at 49–60 °C (depending on the primer) for 30 s, elongation at 72 °C for 50 s; final elongation at 72 °C for 12 min.

[4] *Zhou Zh., Jiang Y., Wang Zh., Gou Zh., Lyu J., Li W.* Resequencing 302 wild and cultivated accessions identifies genes related to domestication and improvement in soybean // Nature Biotechnology, 2015, vol. 33, N 4, pp. 408–414.

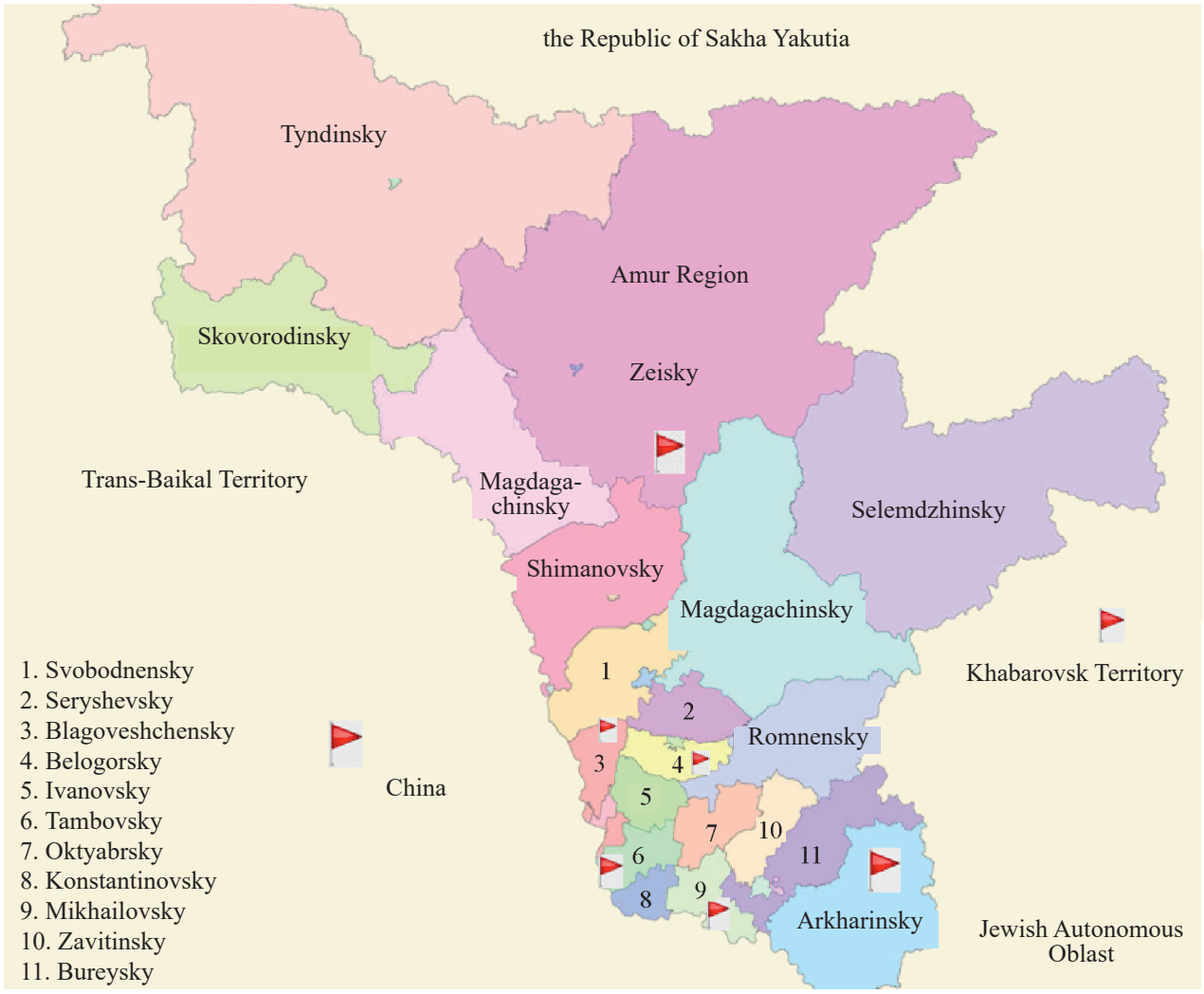

Рис. 1. Места сбора коллекционных форм дикой сои (обозначены красным флажком)

Fig. 1. Collection sites for wild soybeans (marked with a red flag)

Рис. 2. Коллекция *G. soja* на опытном поле ФНЦ ВНИИ сои

Fig. 2. The *G. soja* collection on the FRC ARSRI of Soybean experimental field

The reaction products were separated by electrophoresis in 2% agarose gel stained with ethidium bromide and in 0.5× TBE buffer using a SE-1 horizontal electrophoresis chamber (Helikon, Russia) for 2 h at a current of 50 mA and a voltage of 85 V. Visualization was performed by irradiating the gel with ultraviolet light using the Gel Doc EZ gel-documenting system (Bio-Rad Laboratories Inc., USA). Identification and determination of the sizes of alleles of microsatellite loci were performed using the Image Lab Version 6.0.14 Standard Edition program. The size of the fragments was determined relative to the molecular weight marker 50bp DNA Ladder (ZAO Evrogen, Russia). The identified alleles for each locus were designated by the numbers corresponding to the approximate molecular weight (bp). The absence of an amplified fragment on the electrophoregram was designated as "0".

RESULTS AND DISCUSSION

Previous studies have shown that 20 pairs of primers flanking the Satt236 and Satt590 loci did not hybridize with the matrix DNA, while the rest were monomorphic. The results of the bioinformatics search for associations indicated that not all loci from the 20 markers can be used for genotyping certain qualities that are linked to adjacent regions (QTLs) associated with the necessary traits, such as vegetation period, seed weight, protein and oil content in seeds [12]. Thus, only 12 SSR loci were fully suitable for further genotyping (see Table 1).

One measure of genetic diversity is the number of alleles in a population. The number of alleles is easy to measure, but its disadvantage is that it depends on the size of the sample used to estimate diversity. On the one hand, the greater the number of alleles in a population and the more evenly they are distributed, the more variable the genetic potential of the population is. On the other hand, the greater the number of alleles in SSR loci, the more informative they are for characterizing the population[5]. The total number of alleles identified in a series of analyses was 128 for 12 SSR loci, with 5 to 16 alleles identified for each locus (see Table 1). The average number of alleles per locus was 10.67.

Considering that the level of polymorphism is essentially an indicator of alleles effectively operating in the population (ne), this value correlates with the number of alleles identified in each of the studied loci (i.e. the more alleles identified, the higher the level of polymorphism). The average indicator of the level of polymorphism was 7.04 units.

In addition, the value of the informative value of the markers used (PIC) was calculated. The higher the PIC value for a given locus, the more informative it is as a marker. The following division was adopted depending on the PIC value: PIC > 0.5 - the locus is very informative; 0.5 > PIC > 0.25 - quite informative; PIC < 0.25 - little informative. This indicator characterizes the discriminatory power of the locus not only by the number of alleles detected, but also by

Табл. 1. Характеристика исследуемых микросателлитных локусов

Table 1. Characteristics of the investigated microsatellite loci

Locus	Molecular weight, b.p.	Number of alleles (n_a)	Effective number of alleles (n_e)	The magnitude of information polymorphism (PIC)
Satt470	222…255	5	3,58	0,72
Satt556	173…200	6	3,48	0,71
Satt281	163…200	9	5,62	0,82
Satt005	215…280	13	10,04	0,90
Satt002	115…166	10	5,48	0,82
Satt517	188…256	10	7,13	0,86
Satt442	200…325	14	10,79	0,91
Satt431	150…220	15	8,31	0,88
Satt588	103…134	6	3,42	0,71
Satt373	212…330	9	6,49	0,85
Satt173	240…326	16	10,53	0,91
Satt234	217…310	15	9,67	0,90
Average	103…330	10,67	7,04	0,83

[5]*Backeljau T., De Bruyn L., De Wolfe H., Jordaens K., Van Dongen S., Winnepenninckx B.* Multiple UPGMA and neighbor-joining trees and the performance of some computer packages // Molecular Biology and Evolution, 1996, vol. 13, N 2, pp. 309–313.

the relative frequencies of their occurrence. The PIC value approaches one if the locus has many alleles with approximately equal frequencies of occurrence, and is zero if the locus is monomorphic [6]. In our studies, we found that all the studied SSR loci had PIC > 0.5, which indicates their high information content as molecular genetic markers for assessing the degree of genetic relationship. The average PIC value for the studied forms of wild soybean was 0.83. In this case, the studied set of genotypes had a sufficient sample, which affected the polymorphism of microsatellite loci. As a result, high average values of the informative value of the markers used and their level of polymorphism were revealed.

The discriminatory capabilities of the presented marker system were assessed for wild soybean forms. As a result of amplification, intervarietal polymorphic patterns of DNA fragment distribution were obtained for all loci. Unique sets of alleles with differences in more than two loci were obtained for each of the 51 forms. Molecular genetic passports, or so-called genetic formulas of genotypes, were compiled for each variety based on the identified set of alleles of microsatellite loci (see Table 2).

To study the genetic diversity of wild soybeans from the collection of the Federal Research Center "All-Russian Scientific Research Institute of Soybean", unique alleles were identified for each growing region. For wild soybeans from the Zeisky District, most unique alleles were identified for the KZ-578 form (A234, D230, H198, L310); from the Tambov region – for the KT-158 form (A247, D280, F200, G293, H200, L270); from the Belogorsky district – for the KBel 84 (A234, B185, C188, G315, J267) and KBel-30 forms (C200, E120, H175, K331, L304); from the Mikhailovsky district – for the KM-6367 form (C181, F188, G205, H180); from the Arkharinsky district – for the KA-438 form (E150, I110, K300); from the Blagoveshchensky district – for the KBl-91 form (A234, C200, D244, F250, G275, H175, I110, J272, K250). The form of wild soybean from the Khabarovsk Territory is worth noting, in which nine unique alleles were identified (B173, C188, D222, E145, F235, G275, I130, J304, L240). In addition, six unique alleles were identified for wild soybean forms from China: № 5673 – A247, C163, D250, E127, G250, H205; № 5674 – A255, B190, C168, D244, G290, L250.

Genetic distance is a measure of genetic divergence between species, subspecies or populations of the same species. The smaller it is, the greater the genetic similarity. The value of genetic distance can vary from 0 (there are no common alleles between populations) to 1 (allele frequencies in the population are the same). In this case, the genetic distance varies from 1 to 0. If the genetic distance between two populations is 1, then all genes of these populations have alleles that belong to only one of them. Such a distance corresponds to the completion of the gradual divergence of the population of the original species and the final separation of the new species.

We assessed the degree of genetic relationship of the studied soybean genotypes and statistically processed the obtained results using the POPGENE software package. Based on the analysis of the binary matrix, the genetic identity (GI) and genetic distance (GD) of the studied varieties and wild forms of soybeans were calculated. To visualize the detected genetic distances, a dendrogram was constructed using the unweighted pairwise group method analysis (UPGMA) (see Fig. 3).

Analysis of the obtained hierarchical dendrite allowed us to identify several main clusters in the sample of studied wild soybean genotypes, mainly corresponding to the areas of wild soybean growth. It should be noted that 100% of the wild soybean forms from the Mikhailovsky district are found in one cluster, which indicates their high genetic identity. Note that the maximum distance was found between the wild soybean forms from the Zeisky and Belogorsky districts.

CONCLUSION

For the first time, the analysis of genetic diversity and identification of the gene pool of 47 forms of wild soybean from different areas of the Amur Region and 4 forms from the nearest areas (Khabarovsk and Primorsky Territories, China) were carried out using 20 pairs of SSR

Табл. 2. Молекулярно-генетические формулы коллекционных генотипов *G. soja*, полученные с использованием микросателлитного анализа

Table 2. Molecular genetic formulas of *G. soja* collection genotypes obtained using microsatellite analysis

Variety	Formula*	Variety	Formula*
KZ-671	$A_{247}B_{185}C_{200}D_{222}E_{140}F_{235}G_{250}H_{175}I_{103}J_{330}K_{278}L_{280}$	KM-6361	$A_{234}B_{200}C_{194}D_{275}E_{136}F_{356}G_{265}H_{175}I_{110}J_{267}K_{246}L_{266}$
KZ -616	$A_{240}B_{190}C_{200}D_{230}E_{140}F_{240}G_{250}H_{198}I_{124}J_{330}K_{263}L_{276}$	KM-6368	$A_{234}B_{200}C_{194}D_{280}E_{136}F_{250}G_{265}H_{220}I_{110}J_{267}K_{246}L_{266}$
KZ -596	$A_{247}B_{190}C_{200}D_{244}E_{136}F_{235}G_{250}H_{175}I_{124}J_{330}K_{263}L_{258}$	KM-6352	$A_{234}B_{190}C_{194}D_{280}E_{140}F_{245}G_{265}H_{205}I_{110}J_{272}K_{240}L_{266}$
KZ -599	$A_{240}B_{200}C_{200}D_{250}E_{140}F_{235}G_{250}H_{180}I_{130}J_{312}K_{278}L_{258}$	KM-6365	$A_{234}B_{190}C_{194}D_{280}E_{136}F_{235}G_{215}H_{200}I_{110}J_{272}K_{240}L_{258}$
KZ -578	$A_{234}B_{200}C_{200}D_{230}E_{140}F_{228}G_{250}H_{198}I_{124}J_{304}K_{250}L_{310}$	KM-6367	$A_{222}B_{185}C_{181}D_{275}E_{136}F_{188}G_{205}H_{180}I_{110}J_{272}K_{246}L_{258}$
KZ -592	$A_{240}B_{200}C_{176}D_{250}E_{136}F_{235}G_{250}H_{175}I_{130}J_{304}K_{256}L_{296}$	KM-6376	$A_{222}B_{185}C_{200}D_{280}E_{136}F_{245}G_{200}H_{200}I_{110}J_{267}K_{246}L_{222}$
KZ -642	$A_{247}B_{190}C_{181}D_{250}E_{136}F_{240}G_{235}H_{188}I_{103}J_{320}K_{250}L_{280}$	KM-6360	$A_{222}B_{185}C_{194}D_{280}E_{120}F_{245}G_{215}H_{195}I_{110}J_{267}K_{256}L_{222}$
KZ -1209	$A_{247}B_{190}C_{176}D_{244}E_{136}F_{245}G_{235}H_{205}I_{103}J_{320}K_{256}L_{266}$	KM-322	$A_{222}B_{185}C_{194}D_{267}E_{127}F_{245}G_{215}H_{175}I_{110}J_{267}K_{263}L_{258}$
KT-153	$A_{234}B_{200}C_{194}D_{280}E_{155}F_{256}G_{320}H_{195}I_{118}J_{294}K_{313}L_{276}$	KA-346	$A_{240}B_{185}C_{200}D_{230}E_{166}F_{245}G_{240}H_{175}I_{130}J_{267}K_{331}L_{266}$
KT-156	$A_{234}B_{200}C_{181}D_{275}E_{140}F_{256}G_{320}H_{205}I_{134}J_{304}K_{309}L_{250}$	KA-353	$A_{240}B_{190}C_{200}D_{235}E_{166}F_{245}G_{240}H_{180}I_{130}J_{272}K_{331}L_{240}$
KT-221	$A_{234}B_{190}C_{194}D_{285}E_{155}F_{256}G_{320}H_{195}I_{124}J_{304}K_{326}L_{276}$	KA-360	$A_{234}B_{190}C_{200}D_{235}E_{120}F_{245}G_{240}H_{185}I_{130}J_{272}K_{326}L_{228}$
KT-158	$A_{247}B_{200}C_{168}D_{280}E_{140}F_{200}G_{293}H_{200}I_{103}J_{320}K_{226}L_{270}$	KA-377	$A_{234}B_{190}C_{194}D_{235}E_{145}F_{245}G_{240}H_{175}I_{124}J_{272}K_{326}L_{217}$
KT-164	$A_{234}B_{185}C_{168}D_{285}E_{140}F_{256}G_{315}H_{205}I_{103}J_{320}K_{309}L_{245}$	KA-494	$A_{222}B_{190}C_{194}D_{230}E_{140}F_{245}G_{200}H_{160}I_{103}J_{294}K_{313}L_{217}$
KT-165	$A_{234}B_{190}C_{194}D_{295}E_{140}F_{256}G_{315}H_{210}I_{110}J_{320}K_{326}L_{266}$	KA-438	$A_{247}B_{185}C_{200}D_{230}E_{150}F_{200}G_{200}H_{150}I_{110}J_{294}K_{300}L_{266}$
KT-166	$A_{234}C_{194}D_{290}E_{140}F_{256}G_{315}H_{210}I_{110}J_{320}K_{313}L_{266}$	KA-455	$A_{247}B_{185}C_{200}D_{215}E_{140}F_{200}G_{215}H_{165}I_{130}J_{294}K_{331}L_{250}$
KBel-84	$A_{234}B_{185}C_{188}D_{285}E_{140}F_{245}G_{315}H_{205}I_{110}J_{267}K_{326}L_{217}$	KA-444	$A_{247}B_{185}C_{200}D_{215}E_{140}F_{200}G_{215}H_{180}I_{103}J_{294}K_{331}L_{222}$
KBel -107	$A_{222}B_{190}C_{181}D_{290}E_{127}F_{250}G_{320}H_{205}I_{103}J_{272}K_{313}L_{217}$	KBl -77	$A_{247}B_{200}C_{171}D_{222}E_{127}F_{200}G_{265}H_{185}I_{103}J_{294}K_{246}L_{222}$
KBel -50	$A_{247}B_{190}C_{181}D_{280}E_{140}F_{245}G_{320}H_{205}I_{103}J_{272}K_{326}L_{245}$	KBl -91	$A_{234}B_{210}C_{200}D_{244}E_{120}F_{250}G_{275}H_{175}I_{110}J_{272}K_{250}L_{222}$
KBel -116	$A_{255}B_{200}C_{163}D_{275}E_{127}F_{200}G_{320}H_{190}I_{110}J_{272}K_{305}L_{270}$	KBl -90	$A_{247}B_{210}C_{171}D_{222}E_{120}F_{200}G_{250}H_{170}I_{103}J_{294}K_{224}L_{217}$
KBel -113	$A_{255}B_{200}C_{168}D_{275}E_{127}F_{200}G_{290}H_{185}I_{110}J_{283}K_{305}L_{300}$	KBl -93	$A_{247}B_{190}C_{171}D_{230}E_{136}F_{194}G_{205}H_{180}I_{103}J_{294}K_{263}L_{222}$
KBel -156	$A_{255}B_{200}C_{163}D_{280}E_{127}F_{210}G_{325}H_{180}I_{110}J_{283}K_{292}L_{310}$	KBl-5632	$A_{247}B_{190}C_{163}D_{230}E_{145}F_{194}G_{225}H_{180}I_{103}J_{294}L_{217}$
KBel -1275	$A_{247}B_{190}C_{163}D_{267}E_{115}F_{210}G_{325}H_{180}I_{110}J_{283}K_{305}L_{310}$	KBl -5625	$A_{247}B_{190}C_{181}D_{222}E_{160}F_{194}G_{200}H_{185}I_{103}J_{304}K_{246}L_{217}$
KBel -30	$A_{247}B_{190}C_{200}D_{267}E_{120}F_{210}G_{325}H_{175}I_{110}J_{272}K_{331}L_{304}$	KBl -5618	$A_{247}B_{190}C_{181}D_{215}E_{166}F_{194}G_{200}H_{190}I_{103}J_{304}K_{246}L_{217}$
No. 5673	$A_{247}B_{210}C_{163}D_{250}E_{127}F_{188}G_{250}H_{205}I_{103}J_{320}K_{326}L_{258}$	KBl -5630	$A_{247}B_{185}C_{181}D_{222}E_{166}F_{200}G_{200}H_{185}I_{103}J_{212}K_{240}L_{217}$
No. 5674	$A_{255}B_{190}C_{168}D_{244}E_{136}F_{188}G_{290}H_{200}I_{103}J_{320}K_{309}L_{250}$	No.5683	$A_{234}B_{210}C_{220}D_{285}E_{136}F_{188}G_{235}H_{200}I_{103}J_{320}K_{326}L_{258}$
		Khab-1	$A_{234}B_{173}C_{188}D_{222}E_{145}F_{235}G_{275}H_{200}I_{130}J_{304}K_{309}L_{240}$

*Locus codes: A – *Satt*470, B – *Satt*556, C – *Satt*281, D – *Satt*005, E – *Satt*002, F – *Satt*517, G – *Satt*442, H – *Satt*431, I – *Satt*588, J – *Satt*373, K – *Satt*173, L – *Satt*234. The subscript is the allelic state of the locus (bp).

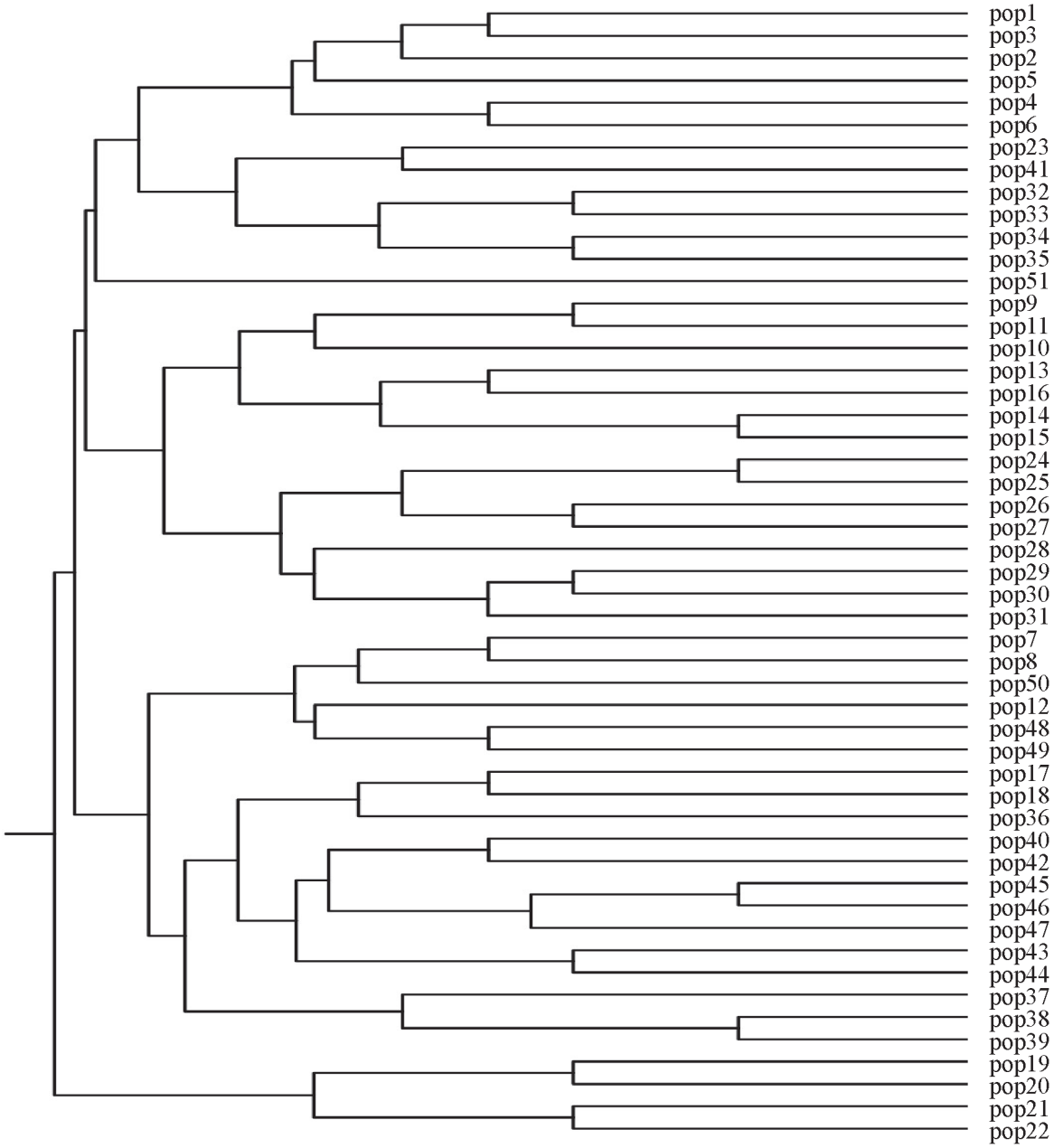

Рис. 3. Дендрограмма, демонстрирующая генетическую близость и отдаленность 51 формы дикой сои:

pop1 – КЗ-671, pop2 – КЗ-616, pop3 – КЗ-596, pop4 – КЗ-599, pop5 – КЗ-578, pop6 – КЗ-592, pop7 – КЗ-642, pop8 – КЗ-1209, pop9 – КТ-153, pop10 – КТ-156, pop11 – КТ-221, pop12 – КТ-158, pop13 – КТ-164, pop14 – КТ-165, pop15 – КТ-166, pop16 – КБел-84, pop17 – КБел-107, pop18 – КБел-50, pop19 – КБел-116, pop20 – КБел-113, pop21 – КБел-156, pop22 – КБел-1275, pop23 – КБел-30, pop24 – КМ-6361, pop25 – КМ-6368, pop26 – КМ-6352, pop27 – КМ-6365, pop28 – КМ-6367, pop29 – КМ-6376, pop30 – КМ-6360, pop31 – КМ-322, pop32 – КА-346, pop33 – КА-353, pop34 – КА-360, pop35 – КА-377, pop36 – КА-494, pop37 – КА-438, pop38 – КА-455, pop39 – КА-444, pop40 – КБл-77, pop41 – КБл-91, pop42 – КБл-90, pop43 – КБл-93, pop44 – КБл-5632, pop45 – КБл-5625, pop46 – КБл-5618, pop47 – КБл-5630, pop48 – № 5683, pop49 – № 5673, pop50 – № 5674, pop51 – Хаб-1

Fig. 3. A dendrogram showing the genetic proximity and remoteness of 51 forms of wild soybeans:

pop1 – KZ-671, pop2 – KZ-616, pop3 – KZ-596, pop4 – KZ-599, pop5 – KZ-578, pop6 – KZ-592, pop7 – KZ-642, pop8 – KZ-1209, pop9 – KT-153, pop10 – KT-156, pop11 – KT-221, pop12 – KT-158, pop13 – KT-164, pop14 – KT-165, pop15 – KT-166, pop16 – KBel-84, pop17 – KBel-107, pop18 – KBel-50, pop19 – KBel-116, pop20 – KBel-113, pop21 – KBel-156, pop22 – KBel-1275, pop23 – KBel-30, pop24 – KM-6361, pop25 – KM-6368, pop26 – KM-6352, pop27 – KM-6365, pop28 – KM-6367, pop29 – KM-6376, pop30 – KM-6360, pop31 – KM-322, pop32 – KA-346, pop33 – KA-353, pop34 – KA-360, pop35 – KA-377, pop36 – KA-494, pop37 – KA-438, pop38 – KA-455, pop39 – KA-444, pop40 – KBl-77, pop41 – KBl-91, pop42 – KBl-90, pop43 – KBl-93, pop44 – KBl-5632, pop45 – KBl-5625, pop46 – KBl-5618, pop47 – KBl-5630, pop48 – no. 5683, pop49 – no. 5673, pop50 – no. 5674, pop51 – Hub-1

markers, the degree of DNA polymorphism by microsatellites was assessed, based on which genetic formulas of genotypes were compiled for the purpose of molecular genetic certification and differentiation of wild soybean forms. A dendrogram of genetic similarity was constructed based on SSR analysis, the degree of genetic relationship of the studied forms of wild soybean from the collection of the Federal Research Center "All-Russian Scientific Research Institute of Soybean" was established. It was shown that the maximum number of unique alleles (nine each) was identified among the forms from the Blagoveshchensk District (KBl-91) and Khabarovsk Territory (Khab-1). Wild soybean forms from the Arkharinsky District were distinguished by insignificant genetic diversity (no more than three unique alleles for each).

In breeding programs, it is recommended to use forms with the greatest diversity of unique alleles. Thus, the marker system tested in the work can be used for genetic differentiation of wild soybean forms.

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Реализация потенциала продуктивности крамбе абиссинской при различном уровне питания

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Представлены результаты исследования влияния предпосевного внесения различных доз минеральных удобрений на продуктивность крамбе абиссинской в условиях лесостепи Среднего Поволжья. Исследования проводили в 2021–2023 гг. в Пензенской области. Вегетационный период крамбе в 2021 г. характеризовался недостаточным увлажнением (ГТК 0,80). В 2022 г. условия характеризовались обильным увлажнением (ГТК 1,29). Условия 2023 г. были наиболее благоприятными для развития крамбе (ГТК 1,02). Показано, что улучшение уровня питания способствует повышению полевой всхожести на 2,8–9,2%, сохранности растений к уборке – на 1,4–7,1%. Максимальный (91,6%) показатель полноты всходов получен при сочетании $N_{30}P_{30}K_{30}$. Наибольшее увеличение сохранности растений отмечено в вариантах с применением $N_{30}P_{30}K_{30}$ (98,6%) и $N_{30}P_{60}K_{60}$ (97,2%), что на 7,1 и 5,7% соответственно превышает контроль. Число плодиков на одном растении в зависимости от доз удобрений изменялось от 852,6 до 995,4 при 819,4 в контрольном варианте. Наибольшая (5,05 г) масса семян с одного растения отмечена на фоне $N_{30}P_{60}K_{60}$. В вариантах с использованием $N_{30}P_{30}K_{30}$ и $N_{30}P_{60}K_{30}$ получены наиболее крупные семена, масса 1000 семян которых составила 8,81 и 8,91 г. Наиболее мелкие семена отмечены в вариантах при внесении $N_{30}P_{30}K_{60}$ (7,73 г) и $N_{60}P_{30}K_{60}$ (7,74 г). Предпосевное внесение удобрений способствовало увеличению урожайности культуры до 1,95–2,13 т/га, прибавка составила 0,03–0,21 т/га. Максимальная урожайность получена в вариантах с внесением $N_{30}P_{60}K_{30}$ и $N_{30}P_{30}K_{30}$ – 2,13 и 2,10 т/га соответственно. Наибольшая масличность семян отмечена на фоне с применением $N_{30}P_{60}K_{60}$ (37,6%) и $N_{30}P_{30}K_{30}$ (36,5%). В вариантах с внесением N_{60} в различных сочетаниях масличность семян снижалась на 0,2–2,7% по отношению к фону N_{30}. Применение минеральных удобрений снижает содержание эруковой кислоты до 60,3–60,9%. Исключение составил вариант $N_{30}P_{60}K_{60}$, где данный показатель был максимальным – 62,5%.

Ключевые слова: крамбе абиссинская, минеральные удобрения, урожайность, масличность, жирнокислотный состав, структура урожая

Realization of productivity potential of Crambe Abyssinica at different nutritional levels

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The results of the study of the influence of pre-sowing application of different doses of mineral fertilizers on the productivity of Crambe Abyssinica in the conditions of the forest-steppe of the Middle Volga region are presented. The research was conducted in 2021– 2023 in the Penza region. The growing season of crambe in 2021 was characterized by insufficient moisture (HTC 0.80). In 2022, the conditions were characterized by abundant moisture (HTC 1.29). The 2023 conditions were most favorable for crambe development (HTC 1.02). It is shown that improvement of nutrition level contributes to the increase of field germination by 2.8–9.2%, plant viability to harvesting by 1.4–7.1%. The maximum (91.6%) indicator of sprouting completeness was obtained with the combination of $N_{30}P_{30}K_{30}$. The greatest increase in plant viability was observed in the variants with application of

$N_{30}P_{30}K_{30}$ (98.6%) and $N_{30}P_{60}K_{60}$ (97.2%), which is 7.1 and 5.7% higher than the control, respectively. The number of fruits on one plant depending on the fertilizer doses varied from 852.6 to 995.4 with 819.4 in the control variant. The highest (5.05 g) seed weight per plant was observed on the background of $N_{30}P_{60}K_{60}$. The variants using $N_{30}P_{30}K_{30}$ and $N_{30}P_{60}K_{30}$ produced the largest seeds with 1000 seed weight of 8.81 and 8.91 g. The smallest seeds were observed in the variants when $N_{30}P_{30}K_{60}$ (7.73 g) and $N_{60}P_{30}K_{60}$ (7.74 g) were applied. Pre-sowing fertilizer application increased the crop yield to 1.95–2.13 t/ha, the increment was 0.03–0.21 t/ha. Maximum yield was obtained in the variants with application of $N_{30}P_{60}K_{30}$ and $N_{30}P_{30}K_{30}$ – 2.13 and 2.10 t/ha, respectively. The highest oil content of the seeds was observed on the background with application of $N_{30}P_{60}K_{60}$ (37.6%) and $N_{30}P_{30}K_{30}$ (36.5%). In the variants with the application of N_{60} in various combinations oil content of the seeds decreased by 0.2–2.7% in relation to the background N_{30}. Application of mineral fertilizers reduced the content of erucic acid to 60.3–60.9%. The exception was the variant $N_{30}P_{60}K_{60}$, where the percentage of erucic acid was the maximum 62.5%.

Keywords: Crambe Abyssinica, fertilizers, doses, yield, oil content, fatty acid composition, crop structure

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INTRODUCTION

Currently, oil-bearing cabbage crops are becoming increasingly important in agricultural production, of which rapeseed, mustard, coleseed and camelina are grown on an industrial scale in many regions of Russia [1]. Recently, Crambe abyssinica has attracted considerable interest from agricultural producers both in terms of economic and agronomic indicators as an alternative to traditional oil crops [2, 3].

Crambe abyssinica H. is a high-margin oilseed crop, the value of which is determined by high seed productivity, as well as the gross yield of vegetable oil [4, 5].

The fruits and seeds of crambe contain 25–35 and 42–46% fatty oil, respectively, with a low iodine value (93–97)[1] [6]. Crambe oil has a high content of erucic acid – up to 60% and higher. The concentration of oleic acid is 13–16%, linoleic – 8–14, linolenic – 8–10% [8]. Various studies show that crambe has high antioxidant activity, since its seeds contain a fairly high content of ascorbic acid (0.36%) and carotenoids (11,52%) [5, 6].

Due to the beneficial properties of the oil, crambe has great potential for use in the chemical and processing industries [7, 9], in medicine and perfumery [8, 10], as a source of biofuel [11, 12], and in the food industry for the preparation of mayonnaise and margarine (see footnote 1) [5]. Numerous tests in various regions have shown it to be an unpretentious and tolerant crop

[1]*Prakhova T.Ya.* Promising oilseed crop Crambe Abyssinica // Achievements of Science and Technology of the AIC, 2013, No. 8, pp. 31–33.

to various environmental conditions [3, 9], as well as a good green manure and phytosanitary crop [4, 13]. Despite this, crambe is considered a rare crop, which is due to the insufficient development of the sales market and the lack of knowledge of the features of its cultivation technology.

Recently, climate change has had an aggravating effect on the productivity of agricultural plants. Often, stress becomes the main factor limiting both their yield and quality. One of the main conditions for increasing crop yield is the use of resource-saving agricultural technologies, including optimization of the nutrition regime [14, 15]. It should be noted that the relationship of crambe to various doses and forms of fertilizers has been insufficiently studied. In this regard, studying the effectiveness of the influence of mineral nutrition on crop productivity in unstable agroecological conditions of the Middle Volga region is an important scientific and production task and determines the relevance of this work.

The purpose of the study is to investigate the effect of different doses of mineral fertilizers on the productivity of Crambe abyssinica in the forest-steppe conditions of the Middle Volga region.

MATERIAL AND METHODS

The studies were conducted in 2021–2023 in the fields of the Penza Research Institute of Agriculture, a branch of the Federal Scientific Center for Bast Fiber Crops. The object of the studies was the Crambe abyssinica of the Demetra variety. The experiments were set up, all crop records and observations were carried out in accordance with the methodological recommendations[2]. The oil content of crambe seeds was determined by the Soxhlet method, protein by the Kjeldahl method, and fatty acid composition by gas-liquid chromatography on a Crystal 5000.1 chromatograph according to GOST R 51483–99 in the agrochemical laboratory of the Penza Research Institute of Agriculture.

The experiment consisted of studying the pre-sowing application of various doses of mineral fertilizers. The experimental design included the following options: control; $N_{30}P_{30}K_{30}$; $N_{30}P_{60}K_{60}$; $N_{30}P_{60}K_{30}$; $N_{30}P_{30}K_{60}$; $N_{60}P_{60}K_{60}$; $N_{60}P_{60}K_{30}$; $N_{60}P_{30}K_{30}$; $N_{60}P_{30}K_{60}$; carbamide.

The sowing of crambe was carried out at the optimally early date (first ten days of May), in rows with a seeding rate of 2.5 million viable seeds/ha. Harvesting was carried out by the direct method in the phase of full maturity of the crop using the Sampo-130 selection combine. The area of the plot was 10 m^2, the experiment was repeated three times, the placement of the plots was sequential.

The soils of the experimental plot are represented by medium-deep leached chernozems. The humus content in the arable layer is on average 5.73%, pH_{salt} – 4.9. The amount of easily hydrolyzed nitrogen was 86.1 mg/kg (according to Tyurin and Kononova, GOST 26951–86), available phosphorus – 132.1 mg/kg (according to Chirikov, GOST 26204–91), exchangeable potassium – 107.7 mg/kg (according to Chirikov, GOST 26204–91).

The main agroclimatic indicators during the vegetation period of crambe over the years of research were contrasting (see Table 1).

The vegetation period of crambe in 2021 proceeded in conditions with insufficient moisture, the hydrothermal coefficient was 0.80. A total of 144.5 mm of precipitation fell, or 84% of the average long-term norm. At the same time, average daily temperatures reached 21.2 °C, which is 1.6 °C higher than the climatic norm. In 2022, the crop developed in conditions of abundant moisture (HTC 1.29) with low average daily temperatures (17.0 °C). During the period of active vegetation of the crop, 182.4 mm of precipitation fell, the sum of effective temperatures was only 1411.5 °C with an average of 1810.0 °C. The conditions of 2023 were the most favorable for the development of crambe and were characterized as moderately humid (HTC 1.02). The total precipitation was 169.6 mm and the temperature optimum reached 18.0 °C with average

[2]Methodology for conducting field and agrotechnical experiments with oil crops. Krasnodar: VNIIMK, 2010, 323 p.

Табл. 1. Метеорологические показатели за период вегетации крамбе
Table 1. Meteorological indicators for the crambe growing season

Indicator	2021	2022	2023	Long-time average annual data
Sum of temperatures ≥ 10 °C	1803,4	1411,5	1656,0	1810,0
Average daily temperature, °C	21,2	17,0	18,0	19,6
Total precipitation, mm	144,5	182,4	169,6	172,1
HTC	0,80	1,29	1,02	1,10

long-term data of 172.1 mm and 19.6 °C, respectively.

RESULTS AND DISCUSSION

Despite the positioning of Crambe abyssinica as an undemanding crop, studies have shown that it responds positively to the application of mineral fertilizers.

Fertilizer application before sowing has a certain effect on the initial changes occurring in the seeds, as well as on the subsequent stage of crambe plant development and, as a consequence, on its productivity as a whole. On average, over the years of research, pre-sowing fertilizer application contributed to an increase in the growth force of crambe seedlings by 0.02–0.17 cm, and in roots by 0.08–0.46 cm relative to the variant without fertilizers (see Table 2).

In the variants with the use of complex fertilizers in the dose of $N_{30}P_{60}K_{60}$ and $N_{30}P_{60}K_{30}$, the strongest sprouts were noted, the length of which was 1.63 and 1.56 cm, which is 0.17 and 0.10 cm higher than the control. However, the "weight of 100 sprouts" indicator was the highest in the $N_{30}P_{30}K_{30}$ variant – 18.7 g with an average sprout length of 1.52 cm. In this variant, despite the fact that the crambe sprouts were shorter compared to other variants, they were stronger and denser, which affected their weight. At the same time, in this variant, the greatest length of roots was noted – 5.31 cm, which significantly (by 0.46 cm) exceeded the indicators of the control variant.

Табл. 2. Сила роста, полевая всхожесть и сохранность растений крамбе в зависимости от доз удобрений (2021–2023 гг.)
Table 2. Growth vigor, field germination and viability of crambe plants depending on fertilizer doses (2021–2023)

Option	Vigour			Germination	Viability of plants for harvesting
	Length of roots	Length of sprouts	Weight of 100 sprouts, g		
	cm			%	
Control	4,85	1,46	12,5	82,4	91,5
$N_{30}P_{30}K_{30}$	5,31	1,52	18,7	91,6	98,6
$N_{30}P_{60}K_{60}$	5,15	1,63	12,8	85,6	97,2
$N_{30}P_{60}K_{30}$	5,27	1,56	15,4	85,2	93,6
$N_{30}P_{30}K_{60}$	4,98	1,50	12,8	87,6	94,2
$N_{60}P_{60}K_{60}$	5,12	1,48	15,0	90,4	92,9
$N_{60}P_{60}K_{30}$	5,04	1,51	16,1	86,0	96,6
$N_{60}P_{30}K_{30}$	4,93	1,54	15,5	90,4	95,7
$N_{60}P_{30}K_{60}$	5,17	1,48	17,2	90,4	96,1
Среднее	5,09	1,52	15,1	87,7	95,2
Cv, %	3,0	3,4	14,0	3,6	2,4
LSD_{05}	0,29	0,06	1,19	1,28	1,17

The field germination of crambe changed slightly on average throughout the experiment – from 82.4 to 91.6%. At the same time, a tendency towards an increase in germination in fertilized variants by 2.8–9.2% relative to the control was noted.

The maximum (91.6%) indicator of completeness of seedlings was obtained with the combination of $N_{30}P_{30}K_{30}$. In the variants against the background of the interaction of $N_{60}P_{60}K_{60}$, $N_{60}P_{30}K_{30}$ and $N_{60}P_{30}K_{60}$, the germination of crambe plants was 90.4% and was insignificantly lower than the previous one, but significantly (by 8.0%) exceeded the control.

The survival of crambe plants for harvesting was high on average over 3 years (92.9–98.6%) and, depending on the fertilizer dose, increased by 1.4–7.1% relative to the control. The greatest increase in plant survival was noted in the variants using fertilizers in combination with $N_{30}P_{30}K_{30}$ (98.6%) and $N_{30}P_{60}K_{60}$ (97.2%), which is 7.1 and 5.7% higher than the control, as well as 0.6–4.3 and 2.0–5.7% higher than other variants.

When studying the influence of mineral nutrition backgrounds on crambe yield, a special role is played by the parameters of the elements of the yield structure.

The use of complex fertilizers had a positive effect on the formation of productivity elements of crambe. The number of fruitlets on one plant, depending on the feeding regime, varied from 852.6 to 995.4, with 819.4 in the control variant. At the same time, the variability of this feature was insignificant, the variation coefficient was 7.0%. Increasing the phosphorus dose to P_{60} contributed to an increase in the number of fruitlets by 41.7–142.8. Their maximum number was obtained against the background of $N_{60}P_{60}K_{60}$ – 995.4, which significantly (by 176) exceeds the values of this indicator in the control (see Table 3).

The greatest variability was observed in seed productivity of one plant, the variability of which in the experimental variants was 14.2%. The seed weight per plant in the variant without fertilizers was 3.51 g, when fertilizers were applied, seed productivity increased by 0.04–1.54 g depending on the nutrition background. The highest (4.97 and 5.05 g) seed weight per plant was noted in the variants against the background of $N_{30}P_{30}K_{30}$ and $N_{30}P_{60}K_{60}$ and significantly exceeded this indicator in the control variant by 1.46 and 1.54 g with an LSD_{05} of 1.17 g.

Табл. 3. Высота растений и элементы структуры урожая крамбе в зависимости от доз удобрений (2021–2023 гг.)

Table 3. Plant height and elements of the structure of crambe harvest depending on fertilizer doses (2021–2023)

Option	Plant height, cm	Number of carpophyls per plant	Seed weight per plant, g	Weight of 1000 seeds, g
Control	99,1	819,4	3,51	7,54
$N_{30}P_{30}K_{30}$	98,8	926,6	4,97	8,81
$N_{30}P_{60}K_{60}$	99,9	976,1	5,05	7,98
$N_{30}P_{60}K_{30}$	99,7	977,0	3,86	8,91
$N_{30}P_{30}K_{60}$	99,6	862,4	4,15	7,73
$N_{60}P_{60}K_{60}$	111,2	995,4	4,06	7,86
$N_{60}P_{60}K_{30}$	107,5	968,3	3,68	7,99
$N_{60}P_{30}K_{30}$	103,9	852,6	3,55	8,04
$N_{60}P_{30}K_{60}$	105,4	899,2	3,72	7,74
Среднее	102,8	919,7	4,06	8,07
Cv, %	4,3	7,0	14,2	5,9
LSD_{05}	7,2	161,1	0,87	0,74

In the variants using $N_{30}P_{30}K_{30}$ and $N_{30}P_{60}K_{30}$, the largest seeds were obtained, the weight of 1000 seeds of which was 8.81 and 8.91 g. The smallest seeds were noted in the variants with the introduction of $N_{30}P_{30}K_{60}$ (7.73 g) and $N_{60}P_{30}K_{60}$ (7.74 g), which insignificantly (by 0.19 and 0.20 g) exceeded the control variant.

Height is not a direct component of productivity, but is positively related to most of them. The height of crambe plants varied from 98.8 cm in the $N_{30}P_{30}K_{30}$ treatment to 111.2 cm with $N_{60}P_{60}K_{60}$. Increasing the dose of the nitrogen component stimulated the greatest plant growth, where the height of crambe was 102.8–111.2 cm, compared to 98.8–99.9 cm in the treatments with a lower dose of nitrogen.

As a result of the conducted studies, with the improvement of mineral nutrition conditions, an increase in the yield of crambe seeds was noted to varying degrees. In the variants with the use of pre-sowing fertilizer application, the crop yield varied within the range of 1.95–2.13 t/ha, depending on the dosage of mineral elements, the increase was 0.03–0.21 t/ha relative to the control (1.92 t/ha).

The maximum yield (2.13 and 2.10 t/ha) was obtained in the variants with the introduction of $N_{30}P_{60}K_{30}$ and $N_{30}P_{30}K_{30}$ under pre-sowing cultivation, which significantly (by 0.21 and 0.18 t/ha) exceeded the yield of the crop without fertilizers (see Table 4).

The lowest productivity was noted in the $N_{60}P_{30}K_{30}$ (1.95 t/ha), $N_{30}P_{30}K_{60}$ (1.95 t/ha) and $N_{60}P_{60}K_{30}$ (1.97 t/ha) variants, where the increase in yield compared to the variant without fertilizers was insignificant and amounted to only 0.03 and 0.05 t/ha with an LSR of 0.11 t/ha.

An important criterion for assessing the effectiveness of fertilizer application is the yield of fat and protein per unit area. On average, over 3 years, the oil content of crambe seeds was 34.8–37.6%, with an increase in fat content depending on the doses of nitrogen in mineral fertilizers.

Experience has shown that in the variants with the introduction of N_{60} in various combinations, the oil content of seeds decreases by 0.2–2.7% in relation to the N_{30} background: 36.1–37.6% versus 34.9–35.9%.

Табл. 4. Урожайность и качество семян крамбе в зависимости от доз удобрений (2021–2023 гг.)

Table 4. Productivity and quality of crambe seeds depending on the fertilizer doses (2021–2023)

Option	Yield, t/ha	Deviation, ±	Oil content, %	Protein content, %
Control	1,92	–	34,8	21,39
$N_{30}P_{30}K_{30}$	2,10	0,18	36,5	22,13
$N_{30}P_{60}K_{60}$	1,99	0,07	37,6	22,87
$N_{30}P_{60}K_{30}$	2,13	0,21	36,3	22,50
$N_{30}P_{30K6}0$	1,95	0,03	36,1	22,18
$N_{60}P_{60}K_{60}$	2,06	0,14	35,9	24,71
$N_{60}P_{60K3}0$	1,97	0,05	35,0	25,08
$N_{60}P_{30}K_{30}$	1,95	0,03	35,1	24,36
$N_{60}P_{30}K_{60}$	2,01	0,09	34,9	23,60
LSD_{05}	0,11	–	1,15	1,50

When assessing the effect of the phosphorus component, an inverse relationship was noted: increasing the phosphorus dose to 60 kg a.i./ha led to an increase in oil content by 0.1–1.1%. If in the variants with P_{30} the oil content was 34.9–36.5%, then with the introduction of P_{60} it increased to 35.0–37.6%. The highest fat content was observed in the background with the use of $N_{30}P_{60}K_{60}$ (37,6%) and $N_{30}P_{30}K_{30}$ (36,5%), which was significantly (by 1.7–2.8%) higher than the values in the variant without fertilizers.

The main factor on which the crude protein content in crambe seeds depends is the provision of plants with nitrogen and phosphorus. In our studies, a tendency towards an increase in the protein content in crambe seeds was noted with the introduction of increasing doses of nitrogen (N_{30}–N_{60}) to 23.60–25.08% at 22.13–22.87% in the variants with a dose of N_{30}, the increase was on average 1.47–2.21%. In the variants with an increase in the dose of the phosphorus component to P_{60}, an increase in protein by an average

of 0.4–2.9% was noted. The maximum accumulation of protein was in the variants with the introduction of $N_{60}P_{60}K_{30}$ and $N_{60}P_{60}K_{60}$, which was 3.32 and 3.69% higher relative to the control variant.

The value of oil is determined primarily by its fatty acid composition. Pre-sowing application of fertilizers had little effect on the quality of the oil, in particular on its fatty acid composition. For example, the maximum (14.7%) increase in oleic acid concentration was noted in the variant with $N_{30}P_{30}K_{30}$. A decrease in the content of this acid to 12.8% relative to the control was noted with the application of $N_{60}P_{30}K_{30}$. In the other variants, the oleic acid content was at the level of the control variant – 13.3–13.0% (see Table 5).

The minimum (8.2 and 6.8%) content of linoleic and linolenic acids was noted in the variant with pre-sowing application of $N_{30}P_{60}K_{60}$, which is 0.2% lower than the control and 0.4–1.4 and 0.1–0.7% lower, respectively, than the indicators of other variants. Application of fertilizers in the doses of $N_{60}P_{60}K_{30}$ and $N_{60}P_{30}K_{30}$ contributed to the greatest (9.3 and 9.6%) accumulation of linoleic acid.

The application of mineral fertilizers reduces the content of erucic acid to 60.3–60.9% compared to 61.9% in the control variant. The exception was the variant with the use of $N_{30}P_{60}K_{60}$, where the content of erucic acid was maximum – 62.5%, which is 0.6% higher than the control variant.

The amount of saturated fatty acids varied from 1.6 to 1.8% in the variants with fertilizer, while it was 1.5% in the control variant.

Табл. 5. Содержание основных жирных кислот в маслосеменах крамбе в зависимости от доз удобрений

Table 5. Content of essential fatty acids in crambe oilseeds depending on the fertilizer doses

Option	Acids				
	oleinic	linoleic	linolenic	erucic	saturated
Control	13,9	8,4	7,0	61,9	1,5
$N_{30}P_{30}K_{30}$	14,7	8,7	6,9	60,8	1,7
$N_{30}P_{60}K_{60}$	13,7	8,2	6,8	62,5	1,6
$N_{30}P_{60}K_{30}$	13,9	8,8	7,2	60,5	1,8
$N_{30}P_{30}K_{60}$	13,5	8,9	7,3	60,7	1,8
$N_{60}P_{60}K_{60}$	13,9	8,9	7,2	60,3	1,8
$N_{60}P_{60}K_{30}$	13,3	9,3	7,4	60,5	1,7
$N_{60}P_{30}K_{30}$	12,8	9,6	7,5	60,5	1,8
$N_{60}P_{30}K_{60}$	13,8	8,6	7,2	60,9	1,7

CONCLUSION

Crambe responds positively to the application of mineral fertilizers in various doses and combinations. The most effective are $N_{30}P_{60}K_{30}$ and $N_{30}P_{30}K_{30}$, the use of which allowed obtaining the maximum realization of the yield potential up to 2.13 and 2.10 t/ha, the increase relative to the control variant (without fertilizers) was 0.21 and 0.18 t/ha. The highest oil content of seeds was noted against the background of the use of $N_{30}P_{30}K_{30}$ (36.5%) and $N_{30}P_{60}K_{60}$ (37.6%), and the use of the latter increased the content of erucic acid to 62.5%. Improved nutritional levels contribute to an increase in field germination by 2.8–9.2% and the survival of plants for harvesting by 1.4–7.1%, and also have a positive effect on the formation of productivity elements of crambe. In the variants with the introduction of $N_{30}P_{30}K_{30}$ and $N_{30}P_{60}K_{30}$, the largest seeds were obtained, the weight of 1000 seeds of which was 8.81 and 8.91 g.

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Влияние погодных условий Кировской области на хозяйственно ценные признаки озимой ржи

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В результате пятилетних исследований (2019–2023) проанализированы 12 селекционно ценных признаков озимой ржи. Объектом исследования являлись 11 районированных и перспективных сортов озимой ржи селекции Федерального аграрного научного центра Северо-Востока. Проведена оценка гидротермических условий, за период изучения выявлено три года с благоприятными условиями весенне-летней вегетации (2019–2021) и два года с избыточным увлажнением (2022, 2023). С помощью корреляционного анализа рассмотрено влияние агрометеорологических факторов отдельных месяцев весенне-летней вегетации на формирование урожайности озимой ржи. Выявлена высокая отрицательная зависимость урожайности от температурного режима июля: среднемесячной температуры воздуха и суммы эффективных температур (>5 °С) (r = –0,94). Количество осадков вносило существенный негативный вклад в формирование урожайности в фазы весеннего кущения – выхода в трубку (апрель – май) (r = –0,93 и –0,89 соответственно). Проведена оценка вариабельности изучаемых признаков в различных погодно-климатических условиях. К высокостабильным признакам относились натура зерна (CV = 1%) и показатели продуктивности колоса: длина, число колосков, зерен, плотность колоса (CV = 3–4%). Наибольшая изменчивость отмечена у показателя число падения (CV = 43%). Для анализа результатов использован метод главных компонент (PCA). Отмечены неодинаковые факторные нагрузки урожаеобразующих показателей в контрастные годы. Крупность зерна в годы с избыточным увлажнением в большей степени влияла на дисперсию урожайности, чем в годы с оптимальными условиями. Выявлено, что урожайность в большей степени зависела от числа колосков, озерненности, массы зерна с колоса, высоты растений и устойчивости к полеганию. Факторные нагрузки перечисленных показателей определяли большую часть дисперсии урожайности: 45,09% в благоприятный год и 48,25% в годы с избыточным увлажнением. Следовательно, селекционная работа по данным признакам будет эффективнее.

Ключевые слова: озимая рожь (*Secale cereale* L.), урожайность, коэффициент вариации, влияние погодных условий, корреляция, метод главных компонент

Influence of weather conditions of the Kirov region on the economically valuable traits of winter rye

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As a result of five-year research (2019–2023), 12 breeding valuable traits of winter rye were analyzed. The object of the study was 11 released and promising winter rye varieties of the FARC North-East selection. The assessment of hydrothermal conditions was carried out, during the study period 3 years with favorable conditions of spring-summer vegetation (2019–2021) and 2 years with excessive moisture (2022, 2023) were identified. The influence of agrometeorological factors of separate months of spring-summer vegetation on the formation of winter rye yield was considered using correlation analysis. A high negative dependence of yield on July temperature regime: average monthly air temperature and the sum of effective temperatures (>5 °C) (r = –0.94) was revealed. The amount of precipitation made a significant negative contribution to the formation of yields in the phases of spring tillering and booting (April–May) (r = –0.93 and –0.89, respectively). The variability of the studied characteristics under different weather and climatic conditions was assessed. Highly stable characteristics included grain unit (CV = 1%) and spikelet productivity indices: length, number of spikelets, grains, spikelet density (CV = 3–4%). The highest variability was observed in the number of spikelets

(CV = 43%). The principal component analysis (PCA) method was used to analyze the results. Different factor loadings of yield-forming indicators in contrasting years were observed. Grain coarseness in years with excessive moisture had a greater influence on yield dispersion than in the years with optimal conditions. It was revealed that the yield was more dependent on the number of spikelets, ear grain content, grain weight per spikelet, plant height and lodging resistance. The factor loadings of the above-mentioned indices determined most of the yield dispersion: 45.09% in a favorable year and 48.25%, hence, breeding work on these characteristics will be more effective.

Keywords: winter rye (*Secale sereale* L.), yield, coefficient of variation, influence of weather conditions, correlation, principal component method

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INTRODUCTION

Global climate changes observed in recent years on our planet are diverse and manifest themselves not only in fluctuations in the hydrothermal regime, but also in the frequent occurrence of climatic anomalies and extreme weather events [1, 2]. The climate pattern of the Kirov region is the presence of such factors limiting crop yields as uneven distribution of heat and moisture resources throughout the growing season of plants, recurrent frosts in the early spring, increased frequency and intensity of extreme events, etc.[1] [3–5].

The hydrothermal regime has a great influence on the yield of winter rye. According to some literary data, the genotype accounts for 25–40% of the yield increase, the remaining 60–75% is determined by environmental conditions – temperature conditions, moisture supply, intensity of sunlight, duration of daylight hours, etc.[2] [6, 7].

Researcher G.N. Potapova [8] noted that with the improvement of hydrothermal growing conditions, the influence of the "year" factor on the yield decreases by approximately 2 times.

In the conditions of northern agriculture of the Non-Chernozem zone of the European part of the Russian Federation, winter rye plants are often exposed to adverse weather conditions, which lead to a decrease in the productivity of the plant, ear and grain size. In breeding work, preference should be given to the source material that favorably combines resistance to adverse environmental factors with high productivity.

The purpose of the research is to assess the influence of hydrothermal conditions of the spring-summer vegetation period (April – July) on the variability of economically valuable traits of winter rye varieties, as well as to establish the contribution of these traits to the formation of yield.

MATERIAL AND METHODS

The experiment was conducted on the experimental field of the Federal Agricultural Research Center (FARC) of the North-East (Kirov) in 2019–2023.

The soil of the experimental plot is sod-podzolic heavy loamy (humus content is 1.37%, mobile phosphorus is 190 mg/kg, exchangeable

[1] *Perevedentsev Yu.P., Frenkel M.O., Shaimardanov M.Z.* Modern changes in climatic conditions and resources of the Kirov region. Scientific ed. E.P. Naumov. Kazan: Kazan State University, 2010, 242 p.

[2] *Nettevich E.D.* Influence of cultivation conditions and duration on the results of variety evaluation by yield // Bulletin of the Russian Academy of Agricultural Sciences, 2001, N 3, pp. 34–38.

potassium is 221 mg/kg of soil, pH of the salt extract is 4.0). The object of the research was 11 promising and released varieties of winter rye bred by the Federal Agricultural Research Center of the North-East. The experiment was laid out in 6-fold replication on the plots with an accounting area of 10 m^2. Harvesting of the plots was carried out in late July – mid-August using a Sampo-130 combine. Field evaluations and harvest recording were carried out in accordance with the methodology[3].

In laboratory conditions, a structural analysis of yield elements was carried out in 10 plants of each variety in three replicates for the following indicators: spike length, number of spikelets per spike, grain content of the spike, grain weight per spike, weight of 1000 grains. The falling number was determined using the Hagberg-Perten method (GOST ISO 3093–2016) on a Falling Number 1900 device[4].

The grain unit was determined according to GOST 10840–2017[5] on the PH–1M grain-unit scale. The ear density was calculated using the formula

$$\text{Spike density, pcs./10 cm} = \frac{\text{Number of spikelets per ear, pcs.}}{\text{Spike length, cm}} \cdot 10.$$

The index of environmental conditions (Ij) was determined using the method of S.A. Eberhart, W.A. Rusell as presented by V.Z. Pakudin[6]; the hydrothermal coefficient (HTC) using the method of G.T. Selyaninov[7]; the coefficient of variation (CV) using the method of B.A. Dospekhov[8].

The data were processed using the methods of variance and correlation analysis, as well as the principal component analysis (PCA) using the AgCStat add-ins in Microsoft Excel 2016 and Agros 2.07.

RESULTS AND DISCUSSION

The spring-summer vegetation period for winter rye plants is an important period for the formation of vegetative and reproductive organs. To analyze the weather conditions of this period, such indicators as the amount of precipitation per month, average monthly air temperatures, hydrothermal coefficient, and also the index of environmental conditions based on the yield feature were used. The years of research differed significantly in temperature conditions and the amount of precipitation (see Table 1).

Табл. 1. Основные метеорологические показатели весенне-летней вегетации озимой ржи (апрель – июль)

Table 1. Basic meteorological parameters of spring-summer vegetation of winter rye (April-July)

Month	Year				
	2019	2020	2021	2022	2023
Average monthly air temperature, ºC					
April	3,8	2,1	5,2	4	6,8
May	13,6	12,2	15,0	8,5	13,8
June	15,8	15,3	19,9	16,1	14,1
July	16	20,5	19,2	20	18,7
Sum of effective temperatures (>5 ºC)					
April	26	8	35	38	81
May	270	225	309	120	279
June	324	308	448	332	272
July	342	481	440	464	423
Total precipitation, mm					
April	16	89	48	63	30
May	38	89	58	53	39
June	94	41	63	118	31
July	57	100	92	130	180
$HTC_{\text{April – July}}$	1,29	1,23	1,17	2,14	1,75
Environmental Conditions Index	1,40	–1,24	–0,32	–0,09	0,24

[3]*Fedin M.A.* Methodology of state variety testing of agricultural crops. Moscow, 1985, 269 p.

[4]GOST ISO 3093–2016. Grain and its processed products. Determination of the falling number by the Hagberg–Perten method. Moscow: Standartinform, 2019, 16 p.

[5]GOST 10840–2017. Grain. Method for determining the grain unit. Moscow: Standartinform, 2019, 19 p.

6*Pakudin V.Z., Lopatina L.M.* Assessment of ecological plasticity and stability of agricultural crops // Agricultural biology, 1984, N 4, pp. 109–113.

[7]*Selyaninov G.T.* Methodology of agricultural climate characteristics // World agroclimatic reference book. L.: Gidrometeoizdat, 1937, pp. 5–29.

[8]*Dospekhov B.A.* Methods of field experiment. Moscow: Agropromizdat, 1985. 351 p.

Analysis of weather conditions over five years of research made it possible to identify three years (2019–2021) with vegetation periods characterized by optimal moisture ($HTC_{April - July}$ = 1.17–1.29) and two years (2022, 2023) with excessive moisture ($HTC_{April - July}$ = 1.75–2.14). According to the environmental conditions index, favorable conditions for the formation of winter rye yields developed in 2019 (Ij = 1.40; average yield in the experiment was 6.12 t/ha). Excessive moisture in April – May 2020 (153–247% of the norm), as well as low temperatures in April ($\Sigma_{ef.\ t>5\ °C}$ = 8 °C) led to the fact that the resumption of rye vegetation in 2020 occurred only on May 3. The late start of the growing season reduced the average yield in the experiment by 43% compared to 2019 (Ij = –1.24; average yield – 3.48 t/ha).

In the conditions of the Kirov region, a reliable negative correlation was established between the yield of winter rye and the amount of precipitation in the critical phases of plant development – spring tillering – booting (April – May) (r = –0.93 ± 0.21 and r = –0.89 ± 0.26, respectively). Excess moisture during this period had a negative impact on the formation of yield. Weather conditions in the flowering and earing phases (June) did not have a significant impact on yield (see Table 2).

A reliable negative correlation was established between the yield and the temperature regime during the grain ripening phase, when there is an active outflow of plastic substances from the leaves and stem to the caryopsis (July) (r = -0.94 ± 0.20). During the ripening of rye grain, the optimal temperature is considered to be 16–20 °C [9]. In 2020, when the lowest yield in the experiment was formed, the deviation of the average monthly temperature in July was 1.6 °C from the norm, and there were also 12 days with an average daily air temperature of more than 20 °C.

Analysis of the main breeding-valuable traits of winter rye over five years made it possible to determine their variability in years with different climatic conditions (see Table 3).

Табл. 2. Влияние основных метеорологических факторов вегетационного периода (апрель – июль) на урожайность сортов озимой ржи

Table 2. Influence of the basic meteorological factors of the vegetation period (April-July) on the yield of winter rye varieties

Correlation feature	Month	Correlation coefficient ($r \pm Sr$)
Total precipitation	April	–0,93* ± 0,21
	May	–0,89* ± 0,26
	June	0,27 ± 0,56
	July	–0,24 ± 0,56
Average daily air temperature	April	0,35 ± 0,54
	May	0,18 ± 0,57
	June	–0,08 ± 0,58
	July	–0,94* ± 0,20
Sum of effective temperatures (>5 °C)	April	–0,31 ± 0,55
	May	0,20 ± 0,57
	June	–0,13 ± 0,57
	July	–0,94* ± 0,20

*Significant at $p \leq 0,05$.

Weather conditions over the five-year period varied, which allowed us to observe the variability of the studied traits. The most stable traits, which vary little depending on the conditions of the year, include the grain unit (CV = 1%) and the productivity indicators of the spike: length, number of spikelets, grains, spike density (CV = 3–4%). The greatest variability by year was noted for the falling number indicator (CV = 43%). The influence of the "year" factor for this trait was 90%, while the influence of the genotype was only 4%. This dependence of the falling number on weather factors is confirmed by numerous studies in other regions of the country[9] [10, 11].

[9] *Ismagilov R.R., Gaysina L.F.* Falling number of winter rye hybrids in the southern forest-steppe conditions of the Republic of Bashkortostan // Vestnik of the Bashkir State Agrarian University, 2013, N. 4, pp. 13–16.

Табл. 3. Сравнительная оценка факторов влияния на варьирование хозяйственно ценных признаков у сортов озимой ржи (г. Киров, 2019–2023 гг.)

Table 3. Comparative analysis of influence factors on variation of agriculturally valuable characteristics in winter rye varieties (Kirov, 2019–2023)

Indicator	Range of feature values by year	LSD_{05}	CV, %	Influence of the factor, %	
				"year"	"genotype"
Yield, t/ha	3,48…6,12	0,32	20	77	13
Weight of 1000 grains, g	24,6…32,0	0,9	11	86	6
Productive bushiness, pcs.	3,4…5,0	0,8	16	27	15
Lodging resistance, points	3,1…4,8	0,3	16	58	31
Plant height, cm	125,3…155,3	5,7	10	49	40
Ear length, cm	10,5…11,6	0,5	4	30	18
Number of spikelets in an ear, pcs.	32,6…34,6	1,4	3	15	42
Ear grain content, pcs.	51,2…55,8	3,5	4	13	36
Grain weight per ear, g	1,31…1,80	0,16	12	42	23
Ear density, pcs. per 10 cm	28,2…31,0	1,1	4	29	37
Falling number, s	64…239	16	43	90	4
Grain unit, g/l	693…714	4	1	59	21

The yield in the experiment varied quite strongly (CV = 20%) depending on the conditions of the year, the influence of the "year" factor was 77%. Reliable differences were found for all the parameters studied in the experiment. Thus, 2019 can be singled out, when reliable increases were observed in yield (+1.40 t/ha), grain size (+3.1 g) and grain weight per a spike (+0.20 g). Of the 12 parameters studied, the genotype had a strong influence only on 3 parameters – the number of spikelets and grains per a spike and spike density.

Analysis of the obtained data by the principal component analysis (PCA) method allowed us to estimate the influence of individual traits on the formation of the yield of varieties in the years with different moisture levels. The values of the factor loads are shown in Table 4. In the years with favorable weather conditions, four main components were identified that lead to yield variability, which together accounted for 89.6% of the total variability of the resulting feature.

The first component can be characterized as "spike productivity and plant resistance to lodging." In it, the parameters of spike productivity have the greatest load (45.09%): the number of spikelets, grain content, grain weight per spike, as well as plant height and resistance to lodging. The second component consisted of the length of the spike, its density and grain unit, which, in the case of optimal moisture conditions during the period of grain formation and filling, are capable of increasing the yield by 23.09%. Productive tillering prevailed in the third component, explaining 13.25% of the variance in the yield of rye varieties. The fourth component, consisting of grain size, is capable of changing the yield by 8.19%.

In the years with unfavorable hydrothermal conditions of the spring-summer vegetation period, other features influencing the yield dispersion were revealed. In particular, the greatest load was distributed not among four, but among three main components (see Table 4). The greatest share of dispersion (48.25%) in the yield was contributed by lodging resistance, plant height and spike parameters: length, number of spikes, grain content, grain weight per a spike. The second component was made up of spike density and 1000-grain weight. Grain size in the years with excessive moisture had a greater positive load than in the years with optimal conditions. Previously, a number of researchers noted an increase in grain size with an increase in precipitation during the grain filling period [12–14].

Табл. 4. Значения факторных нагрузок урожаеобразующих показателей сортов озимой ржи
Table 4. Values of factor loadings of yield-forming indicators of winter rye varieties

Indicator	Main components						
	F1	F2	F3	F4	F1	F2	F3
	Favorable years (2019–2021)				Years with overwetting (2022, 2023)		
Yield	**0,719**	–0,222	–0,267	0,550	–0,414	**–0,826**	–0,074
Weight of 1000 grains	0,381	0,368	0,570	**0,596**	–0,562	**0,591**	0,074
Productive bushiness	0,537	–0,123	**0,653**	–0,472	–0,576	0,185	**0,589**
Resistance to lodging	**0,902**	–0,063	–0,303	–0,002	**–0,794**	0,097	–0,445
Plant height	**–0,834**	0,059	0,228	0,225	**0,665**	0,215	0,288
Ear length	0,159	**0,898**	0,117	–0,136	**–0,933**	0,197	–0,131
Number of spikelets in a spike	**0,920**	–0,247	0,002	–0,100	**–0,885**	–0,350	0,071
Ear grain content	**0,955**	0,054	–0,022	–0,106	**–0,974**	–0,053	0,057
Weight of grain per ear	**0,738**	0,616	0,211	0,024	**–0,927**	0,174	–0,001
Ear density	0,558	**–0,795**	–0,115	0,015	–0,351	**–0,804**	0,306
Grain unit	0,528	**0,599**	–0,248	–0,018	0,432	–0,364	**–0,730**
Variation, %	45,090	23,094	13,252	8,185	48,245	18,283	14,047
Cumulated variation, %	45,090	68,184	81,436	89,621	48,245	66,528	80,575

The third component was represented by productive tillering and grain unit and determined about 14% of the dispersion. The total value of the accumulated variability of factor loadings of the three principal components was 80.58%, the remaining 19.42% was explained by other indicators that were not recorded in this experiment.

CONCLUSION

The study of released and promising varieties of winter rye in the conditions of the central zone of the Kirov region revealed the level of variability of economically valuable traits with changes in the hydrothermal regime during the spring-summer vegetation period. Since the varieties taken into the study were selected in local conditions and maximally adapted to them, no great variability of traits was noted (CV = 1...20%), with the exception of the falling number indicator, which in our studies and in the works of other authors shows maximum variability in changing growing conditions (CV = 43%).

The dispersion analysis allowed us to identify the share of influence of the year's conditions on the studied economically valuable traits. The influence of the "year" factor in the experiment ranged from 13 to 90%. The greatest influence of external conditions was noted for the following indicators: yield (77%), 1000-grain weight (86%) and falling number (90%).

Based on the analysis using the principal component method, the factor loadings with which the studied indicators are included in the principal components are calculated. In our experiment, the first component included the indicators of spike productivity, plant height and lodging resistance, which were responsible for 45.09% of the yield variance in favorable years and 48.25% in the years with excessive moisture, i.e. these indicators responded equally to changes in environmental conditions. The indicators included in the first, most numerous component are responsible for the majority of yield variability, therefore, selection by these characteristics will be more effective.

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Использование микроконтроллеров для определения гомогенности кормосмеси

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Представлены результаты по определению однородности смеси, полученные физическим методом с использованием микротрейсеров (окрашенных частиц железа). Микротрейсеры – внешние индикаторы, которые используют для определения точности смешивания и уровня контаминации в премиксах и комбикормах. Они представляют собой частицы железа одинакового размера, покрытые нетоксичной пищевой краской. Микротрейсеры стабильны к условиям производства (влажность, температура, давление) и не оказывают отрицательного влияния на органолептические свойства и питательность корма. Цель данной работы – проведение оценки уровня гомогенности кормосмесей для объектов аквакультуры с использованием ферромагнитных микротрейсеров. Экспериментальные исследования проведены на технологической линии смешивания промышленного комбикормового завода в Астраханской области. Применяемые гранулированные системы представляли собой кормовые смеси с различным массовым соотношением отдельных компонентов, различающихся по диаметру и насыпной плотности. Проведены серии тестов для разработки параметров высокого качества смешивания и оценки технических характеристик смесителя на технологической линии производства комбикормов на точность смешивания 1 : 100 000. Микротрейсеры F – красный (количество частиц на 1 г составляло 34 000) добавляли через установку ручного ввода микрокомпонентов непосредственно в бункер над смесителем при производстве форелевого корма 41/26 (ВЭ) 6 мм. Размер партии составлял 800 кг. При помощи микротрейсеров определяли однородность в кормосмеси и готовом корме. Установлено, что в тесте 1 критерий однородности по Гауссу составляет не менее 92% и не более 95% (время смешивания 60 с), в тесте 2 и 3 – выше 95% (время смешивания 90 и 120 с). Тестирование доказало, что значения гомогенности кормосмеси и экструдированного комбикорма находятся в одном интервале. Место отбора не оказывает никакого воздействия на гомогенность.

Ключевые слова: гомогенность, микротрейсеры, кормосмесь, экструдированный комбикорм, микродобавки, время смешивания

The use of microcontrollers to determine feed mixture homogeneity

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The results on the determination of the homogeneity of the mixture obtained by a physical method using microtracers (colored iron particles) are presented. Microtracers are external indicators that are used to determine the mixing accuracy and contamination level in premixes and compound feeds. They are iron particles of the same size, coated with non-toxic, food-grade paint. Microtracers are stable to production conditions (humidity, temperature, pressure) and do not adversely affect the organoleptic properties and nutritional value of the feed. The purpose of the work was to assess the

level of homogeneity of feed mixtures for aquaculture facilities using ferromagnetic microtracers. Experimental studies were carried out on the technological mixing line of an industrial feed mill in the Astrakhan region. The granulated systems used were feed mixtures with different mass ratios of individual components differing in diameter and bulk density. A series of tests to develop high-quality mixing parameters and evaluate the technical characteristics of the mixer on the feed production line for mixing accuracy of 1:100000 were conducted. Microtracers F – red (the number of particles per 1 gram was 34,000) were added through the installation of manual input of micro components directly into the hopper above the mixer during the production of trout feed 41/26 (GE) 6 mm. The batch size was 800 kg. Using microtracers, the uniformity in the feed mixture and the finished feed was determined. It was found that in test 1 the Gauss uniformity criterion is at least 92% and no more than 95% (mixing time 60 seconds), in test 2 and 3 above 95% (mixing time 90 and 120 seconds). The testing has proved that the homogeneity values of the feed mixture and the extruded compound feed are in the same range. The selection site has no effect on homogeneity.

Keywords: homogeneity, microtracers, feed mixture, extruded compound feed, micro additives, mixing time

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INTRODUCTION

Mixing is one of the most important operations in the feed production process [1]. Its purpose is to obtain a completely homogeneous mixture so that the nutrient content in each sample taken is the same[1] [2, 3]. The uniformity in size of the ingredients included in the finished feed can directly affect their final dispersion: when all their physical properties are relatively identical, mixing becomes quite simple [4].

If the physical characteristics of the ingredients vary greatly, problems arise with mixing and separating them. Measuring homogeneity involves assessing the physical, chemical and visual properties of the mixture. This is critical to maintaining the consistency of the product [5–9]. The control ingredients-indicators used (salt, colored millet, barley grain, peas, etc.) cannot guarantee a reliable result, since they are partially destroyed during the mixing process. When it comes to vitamins, mineral supplements and medicinal products, the application rate of which is small, these indicators are generally unacceptable [3, 10].

For homogeneous mixing, microanalogues of the named indicator ingredients are required. Factories that use progressive feed production technologies use ferromagnetic microtracers (MT) as indicators to assess the homogeneity of mixing [11, 12]. Microtracers are external indi-

[1] *Ozherelyeva O., Sytolkin A., Vasilenko L., Danyliv M., Vasilenko O.* Compound feed technology in sturgeon fish aquiculture // Biological Resources Development and Environmental Management: International applied research conference KnE Life Sciences, 2020, pp. 194–202. DOI: 10.18502/kls.v5i1.6047.

cators used to determine the mixing accuracy and contamination level in premixes and compound feeds. They are uniformly sized iron particles coated with non-toxic food paint. Microtracers are stable to production conditions (humidity, temperature, pressure) and do not adversely affect the organoleptic properties and nutritional value of the feed.

The purpose of the work is to assess the level of homogeneity of feed mixtures for aquaculture objects using ferromagnetic microtracers.

MATERIAL AND METHODS

Experimental studies were conducted on a mixing process line of an industrial compound feed plant in the Astrakhan region. The granulated systems used were feed mixtures with different mass ratios of individual components, differing in diameter and bulk density. The objective of the study was to conduct a series of tests to develop high-quality mixing parameters and evaluate the technical characteristics of the mixer on the compound feed production process line for mixing accuracy of 1: 100,000. Microtracers F (red, 34,000 particles/g) were added via a manual microcomponent feeder directly into the hopper above the mixer during the production of trout feed 41/26 (GE) 6 mm. The batch size was 800 kg (batches 1–3) (see Fig. 1). The microtracers were used to determine the homogeneity in the feed mixture and the finished feed.

Spot samples were collected for the microtracer analysis procedure. The results were calculated using the Poisson statistical distribution (discrete type distribution of a random variable; describes the probability of a certain number of events occurring in a certain time interval)[2]. Sampling was carried out from the feed mixer and from the bucket elevator and was carried out in the same way for all batches.

Mixed feed from a mixer: 20 samples weighing approximately 100 g were collected at several control points. The samples were collected from the upper layer using a shovel, and at a depth of 70–80 cm using a probe.

Extruded compound feed: 20 samples weighing approximately 100 g were taken from the bucket elevator. The first sample was taken immediately after the start of the flow, the following samples were taken every 5 sec, and the last sample was taken after 95 sec, when the extruded compound feed flow was almost finished. The sampling was carried out using a shovel.

Microtracers were isolated from a spot sample of the product using a rotating magnet. A small filter was fixed on a rotary detector with a special locking ring. The prepared sample was weighed and loaded into a working rotary detector using a funnel. Magnetic particles remained on the filter paper, while the non-magnetic ones fell into a special compartment of the detector. The funnel was carefully cleaned of feed particles using a brush. The filter with magnetic particles was carefully removed from the detector and demagnetized, then the residue was carefully transferred from the small filter to the large one. The large filter was pre-treated with a developing solution. It was left for several seconds (≈10) to develop the microtracers, then the filter was placed on a hotplate to dry. The finished filters were carefully cleaned of foreign particles and the color spots were counted using the Traco-System on a computer.

The following statistics were calculated to do the interpretation (see Fig. 1):

mean particle count; standard deviation in particle count; coefficient of variation (%); χ^2 – value; probability, p (%); the degree of microtracer recovery (%).

Using the calculated values, the probability of the value χ^2 was determined. In one line we found the number of independent elements (upper horizontal line), in the other one – the determined value of χ^2 (vertical column on the left). At the intersection of the horizontal row and vertical column, a probability value was read in the range from a maximum of 0.999 (99.9%) to a minimum of 0.0005 (less than 0.05%). This is the probability that the value of χ^2 found in the

[2]*Shchegoleva S.A., Titov P.L., Pashchenko M.S.* Statistical methods of quality control and management: distribution laws in physical and technical systems: textbook for universities. Vladivostok, 2024, 56 p.

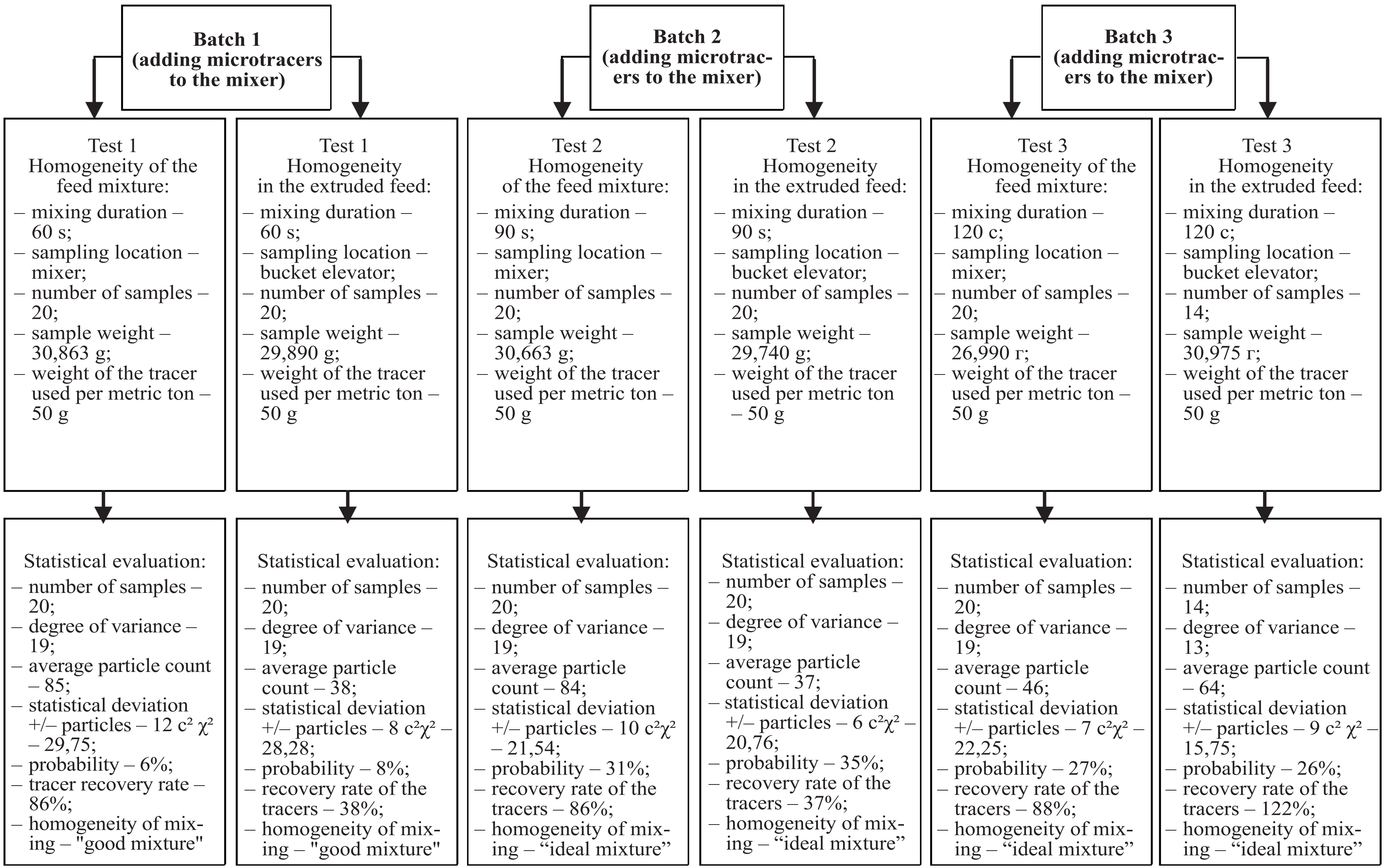

Рис. 1. Схема исследования гомогенности кормосмеси
Fig. 1. The scheme of feed mixture homogeneity research

test will be randomly exceeded by the samples from the "ideal" Poisson mixture.

Numerous studies conducted in the USA, Serbia, Poland, Ireland, Italy, Russia [13] and other countries show the high efficiency and speed of using ferromagnetic tracers to assess the homogeneity of feed (see Table 1).

The results from the Poisson distribution and the homogeneity of mixing *p* are expressed as a probability for comparison and as the coefficient of variation *Cv* from the Gaussian distribution (see footnote 2).

RESULTS AND DISCUSSION

For batch 1, microtracers were added via a manual microadditive injection unit into the hopper above the mixer. 20 samples were collected from the gravity feed after the mixer. Under the test mixing conditions, the mixture achieved a mixing accuracy of 1:100,000 in the mixer with a mixing time of 60 s (see Fig. 2). The calculated probability for microtracers was 12%, which corresponds to a “good” mixture.

Batch 1 – homogeneity of the extruded compound feed. For Batch 1, microtracers were added via a manual feeder into the hopper above the mixer. 20 samples of compound feed were collected from the bucket elevator. Under test conditions, the mixture achieved a mixing accuracy of 1:100,000 at 60 s (see Fig. 3).

The calculated probability for microtracers was 8%, which corresponds to a “good” mixture.

For batch 2, microtracers were added via a manual microadditive injection unit into the hopper above the mixer. 20 samples were collected from the gravity feed after the mixer.

Under test conditions, the mixture achieved a mixing accuracy of 1:100,000 in a mixer at a time of 90 s. The calculated probability for microtracers was 31% (see Fig. 4), which corresponds to an “ideal” mixture.

Batch 2 – homogeneity of the extruded compound feed. For Batch 2, microtracers were added via a manual feeder into the hopper above the mixer. 20 samples of compound feed were collected from the bucket elevator. Under test conditions, the mixture achieved a mixing accuracy of 1:100,000 in a mixture at a time of 90 s

Табл. 1. Определение однородности смешивания, %

Table 1. Determination of mixing uniformity, %

Mixture	Probability, *p*	Coefficient of variation, *Cv*
Ideal	> 25	< 5
Good	> 5 – < 25	> 5 – < 8
Acceptable	> 1 – < 5	> 8 – < 12
Heterogeneous	< 1	> 12

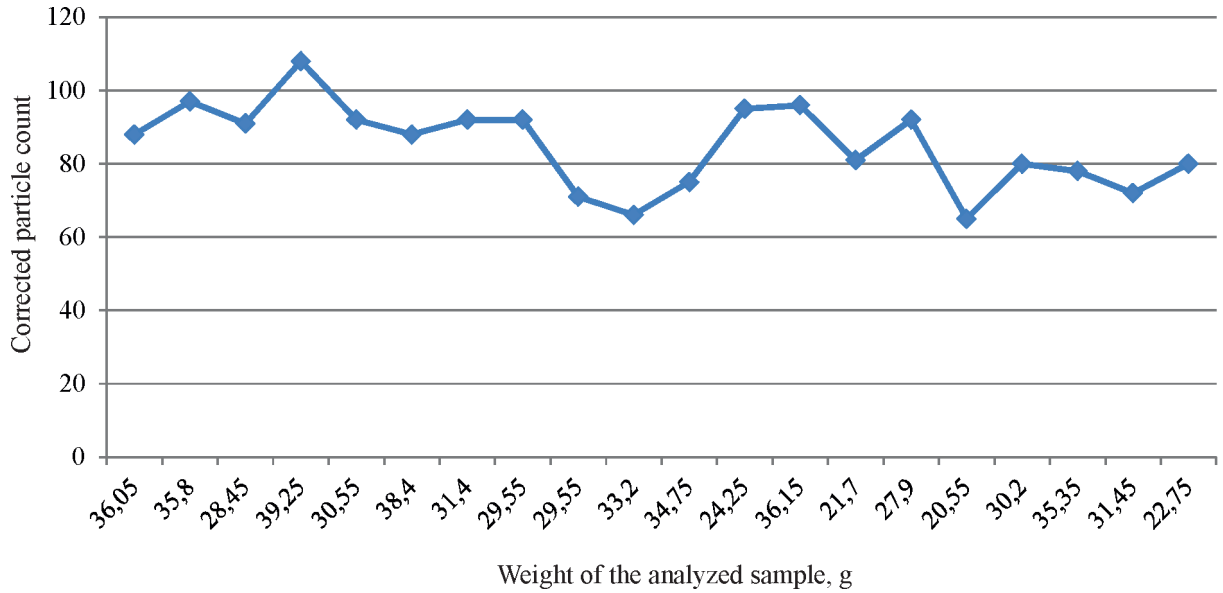

Рис. 2. Гомогенность кормосмеси в смесителе (время перемешивания 60 с)

Fig. 2. Homogeneity of the feed mixture in the mixer (mixing time 60 sec.)

Рис. 3. Гомогенность готового комбикорма (время перемешивания 60 с)
Fig. 3. Homogeneity of the finished compound feed (mixing time 60 sec.)

(see Fig. 5). The calculated probability for microtracers was 35%, which corresponds to an "ideal" mixture.

For batch 3, microtracers were added via a manual microadditive feeder into the hopper above the mixer. 20 samples were collected from the gravity feed after the mixer. All the samples were analyzed for homogeneity. Under test conditions, the mixture achieved a mixing accuracy of 1:100,000 in the mixer at 120 s (see Fig. 6). The calculated probability for microtracers was 27%, which corresponds to an "ideal" mixture.

Batch 3 – homogeneity of the extruded compound feed. For batch 2, microtracers were add-

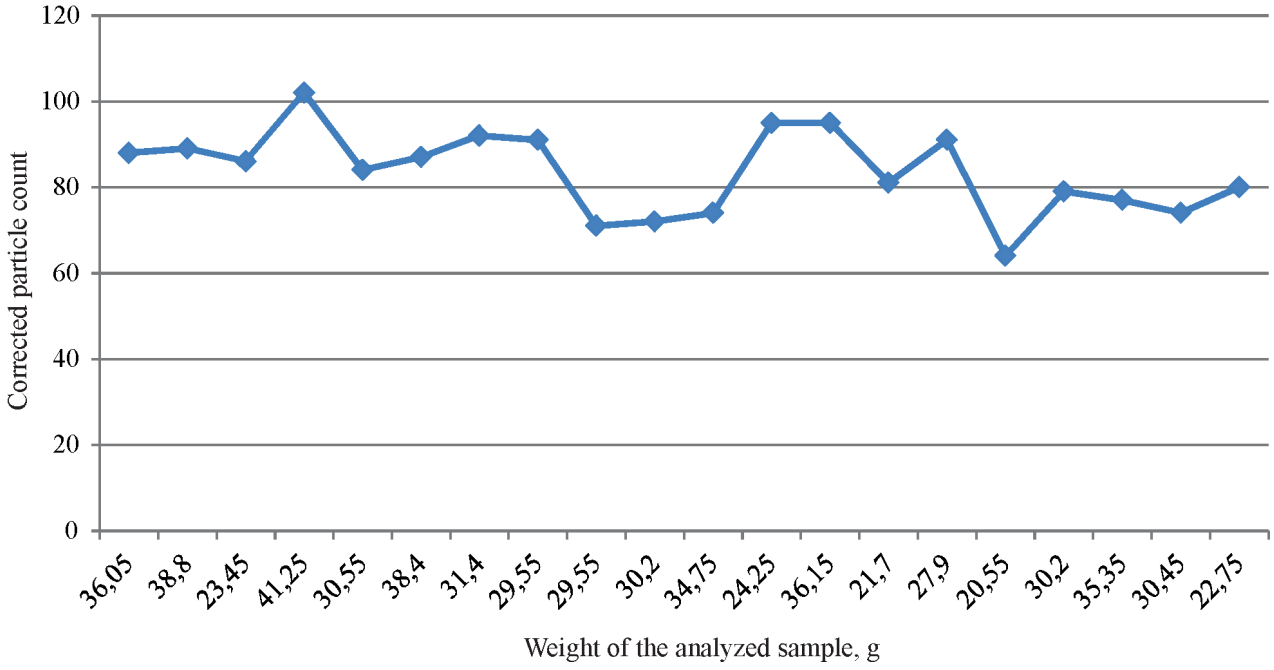

Рис. 4. Гомогенность кормосмеси в смесителе (время перемешивания 90 с)
Fig. 4. Homogeneity of the feed mixture in the mixer (mixing time 90 sec.)

ed via a manual feeder into the hopper above the mixer. 14 samples of the feed were collected from the bucket elevator. All samples were analyzed for homogeneity. Under the test conditions, the mixture achieved a mixing accuracy of 1:100,000 in a mixture at a time of 120 s (see Fig. 7). The calculated probability for microtracers was 35%, which corresponds to an “ideal” mixture.

Table 2 summarizes the results of mixing accuracy tests in the production of extruded compound feed.

The requirements for mixing accuracy and operating precision of the production line (1:100,000) for feed are met in accordance with EU Regulation No 183/2005 (feed hygiene requirements):

– the probability of the feed mixture was 6 > 5% in batch 1 in the mixer, which corresponds to a "good" mixture. Mixing time 60 s;

– the probability was 8 > 5% in batch 1 in the extruded compound feed, which corresponds to a “good” mixture. Mixing time 60 s;

– the probability of the feed mixture was 31> 25% in batch 2 in the mixer, which corresponds to an "ideal" mixture. Mixing time 90 s;

– the probability was 35 > 25% in batch 2 in the extruded compound feed, which corresponds to an "ideal" mixture. Mixing time 90 s;

– the probability of the feed mixture was 27> 25% in batch 3 in the mixer, which corresponds to an "ideal" mixture. Mixing time 120 s;

– the probability was 26 > 25% in batch 2 in the extruded compound feed, which corresponds to an "ideal" mixture. Mixing time 120 s.

The "degree of microtracer recovery, %" indicator is the ratio of the average particle count to the reference average particle count (specified in the certificate for the premix with microtracers, taking into account the microtracer content in the premix, expressed in %). It is considered that the deviation of this indicator from 100% is acceptable by +\–25%. Values within the range of 75–125% are considered normal if the indicators are outside this range. It is necessary to study the operation of the process equipment in more detail during the testing period to identify the causes of the deviation. Microtracers describe the behavior of small particles (vitamins, enzymes, amino acids) inside transport equipment. In our case, in tests 1 and 2, the values of this indicator are very close and are within the permissible limits for the feed mixture. In

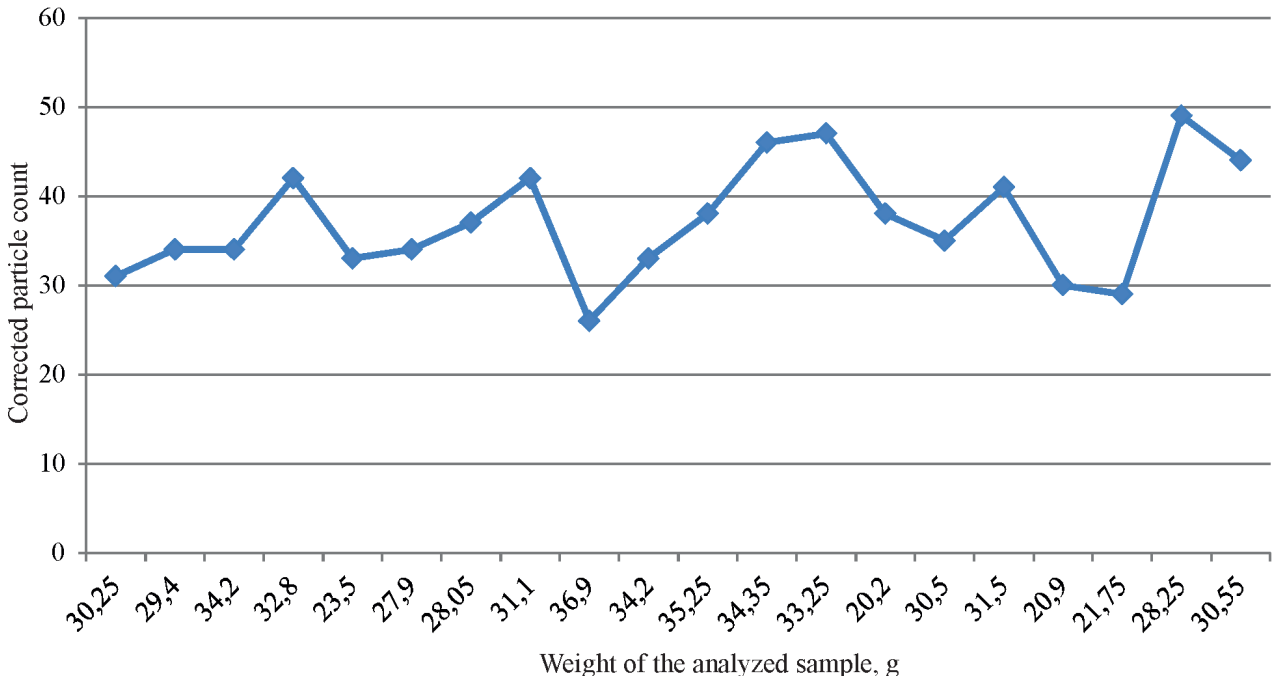

Рис. 5. Гомогенность готового комбикорма (время перемешивания 90 с)

Fig. 5. Homogeneity of the finished compound feed (mixing time 90 sec.)

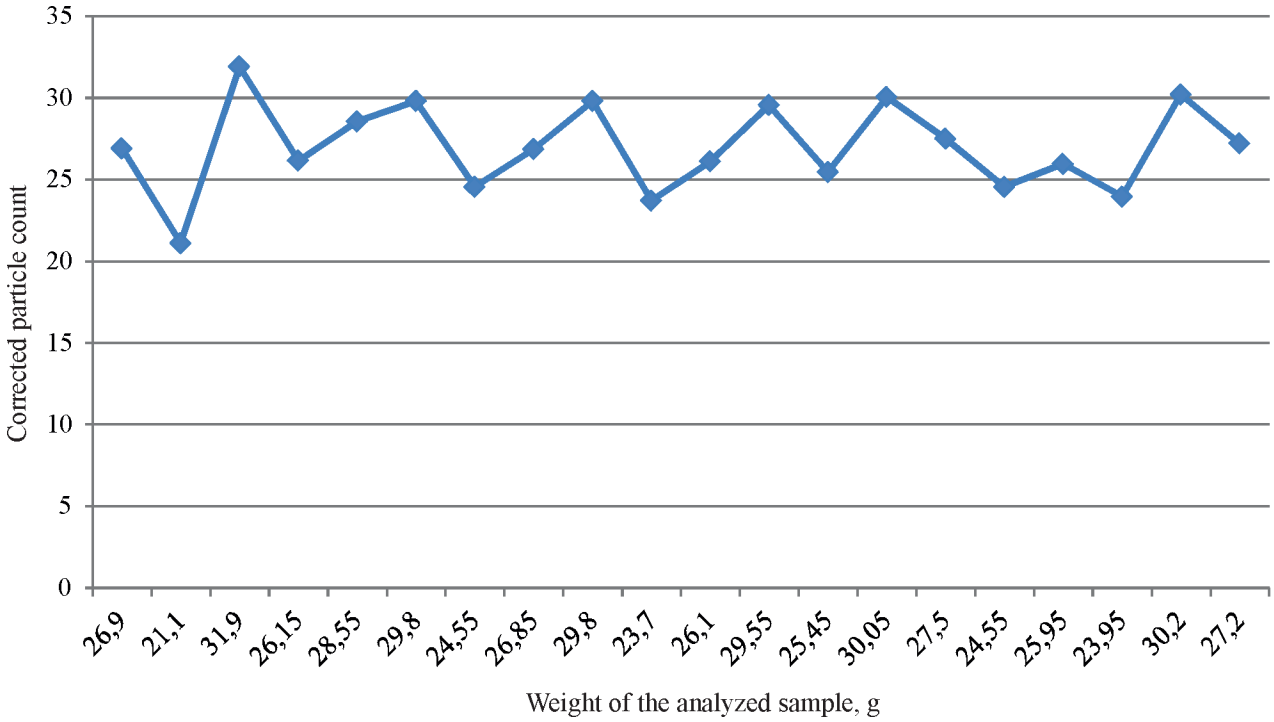

Рис. 6. Гомогенность кормосмеси в смесителе (время перемешивания 120 с)
Fig. 6. Homogeneity of the feed mixture in the mixer (mixing time 120 sec.)

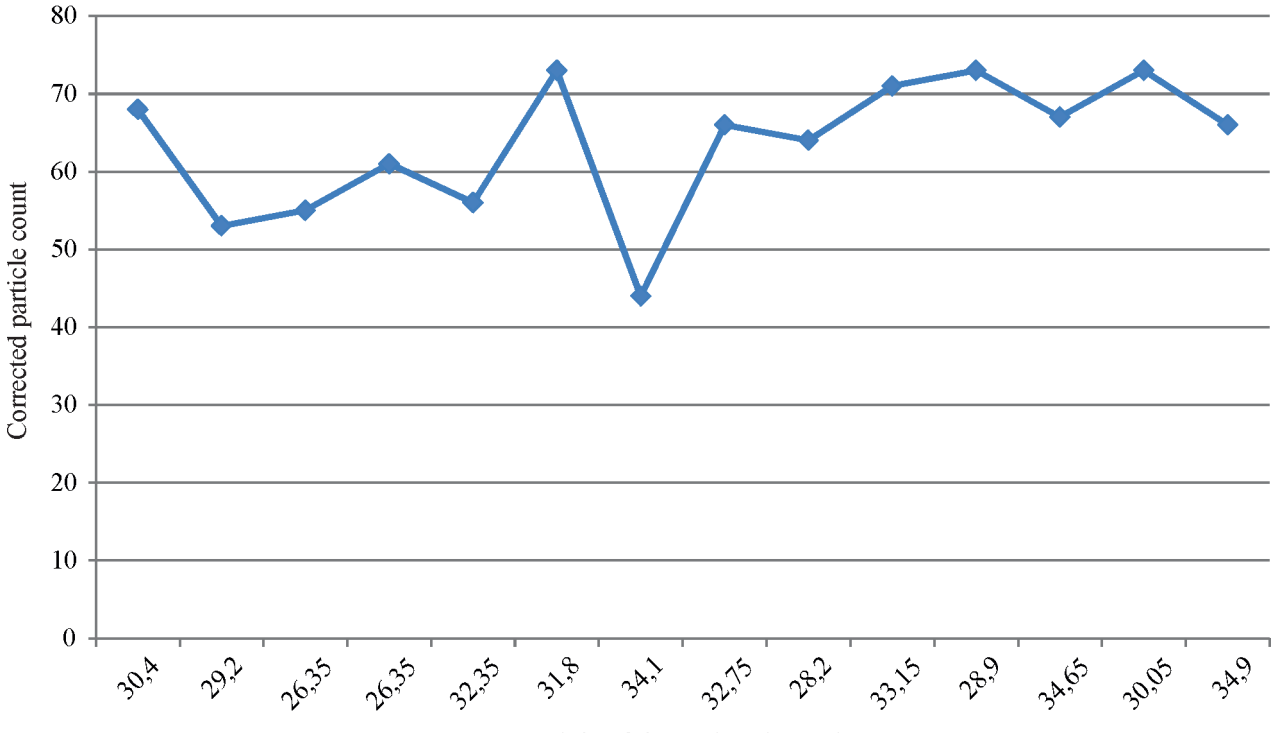

Рис. 7. Гомогенность готового комбикорма (время перемешивания 120 с)
Fig. 7. Homogeneity of the finished compound feed (mixing time 120 sec.)

Табл. 2. Результат тестов на точность смешивания компонентов
Table 2. Test result for accuracy of mixing the components

Batch	Batch size/feed mixture	Feed mixture	Extruded compound feed
Batch 1, test 1; probability, %	800 kg TF 41/26 (GE)	6% (good mixture)	8% (good mixture)
Batch 1, test 1; degree of recovery, %		86%	38%
Batch 2, test 2; probability, %	800 кг TF 41/26 (GE)	31% (ideal mixture)	35% (ideal mixture)
Batch 2, test 2; degree of recovery, %		86%	37%
Batch 3, test 3; probability, %	800 кг TF 41/26 (GE)	27 % (ideal mixture)	26% (ideal mixture)
Batch 3, test 3; degree of recovery, %		88%	122%

the extruded compound feed, the values of this indicator are below the permissible limit, which is presumably caused by the high percentage of fat input, which led to the "dilution" of the feed mixture with microtracers. Test 3 was within the acceptable range for microtracer recovery, but showed an unexpectedly high result in the extruded feed. This is presumably due to a mix-up of the batches (the last batch was less than 800 kg), which led to an incorrect calculation of this indicator.

CONCLUSION

Conclusions on the homogeneity of mixing based on the determination of a specific marker in the samples under study (table salt, phosphorus, calcium, etc.) after statistical data processing are made on the basis of the value of the variation coefficient (*Cv*). In test 1, the Gaussian homogeneity criterion is no less than 92% and no more than 95% (mixing time 60 s), in tests 2 and 3 it is higher than 95% (mixing time 90 and 120 s). Testing has proven that the homogeneity values of the feed mixture and extruded compound feed are in the same range. The sampling location has no effect on homogeneity. Thus, the feed mill process equipment does not subject the feed mixture and finished compound feed to stratification during transportation in the tested area.

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Сорные растения в посевах кукурузы в Белгородской области

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Видовой состав сорных растений в посевах кукурузы, возделываемой в разных регионах Российской Федерации, не идентичен. Это значительно повышает роль регионального фитосанитарного мониторинга посевов, обусловливающего разработку прогноза для принятия решений по выбору конкретных агротехнических и химических мер борьбы с сорными растениями. Выявлен видовой состав сорных растений в посевах кукурузы в Белгородской области и разработан многолетний прогноз их произрастания в данных агрофитоценозах. Материалом для проведения исследования послужили данные фитосанитарного мониторинга состояния посевов кукурузы в Белгородской области в 2020, 2021 гг. Возможность создания регионального прогноза обусловлена масштабом полевых исследований (обследовано 40 полей в разных районах Белгородской области). Достоверность результатов определена использованием апробированной в многолетней практике методики обследования полей. Научное обоснование стабильности произрастания выявленных видов сорных растений на территории Белгородской области обеспечивает разработку регионального прогноза на длительную временную перспективу. Зарегистрировано 32 вида сорных растений, среди которых преобладают малолетние виды (65,63%). Фитосанитарная роль каждого вида выявлена путем определения активности каждого в ценофлоре агрофитоценозов. Группа высокоактивных видов сорных растений включает ежовник обыкновенный, марь белую, щетинник сизый, паслен черный, бодяк седой, гречишку вьюнковую, щирицу назадзапрокинутую. В довольноактивные виды входят вьюнок полевой, осот полевой, спорыш птичий. Группа малоактивных включает 13 видов: полынь обыкновенную, дурнишник обыкновенный, сурепку дуговидную, чистец однолетний, дескурайнию Софии, горошек мышиный, мальву маленькую, сокирки великолепные, циклахену дурнишниколистную, латук компасный, лебеду раскидистую, горец перечный, трехреберник непахучий. Выявлено девять неактивных видов, изредка регистрируемых в посевах кукурузы. Распределение видового состава по группам разного статуса активности будет сохранено в длительной временной перспективе при условии неизменности состояния гидротермических условий, сохранении структуры посевных площадей и соблюдении особенностей региональной технологии возделывания кукурузы и системы защитных мероприятий.

Ключевые слова: мониторинг, активность видов, многолетний региональный прогноз

Weeds in corn crops in the Belgorod region

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The species composition of weeds in corn crops cultivated in different regions of the Russian Federation is not identical. This fact significantly increases the role of regional phytosanitary monitoring of crops, which leads to the development of a forecast for making decisions on the choice of specific agrotechnical and chemical measures to combat weeds. The species composition of weed plants in corn crops in the Belgorod region was identified and a multi-year forecast of their growth in these agrophytocenoses was developed. The data of phytosanitary monitoring of corn crops in the Belgorod

region in 2020, 2021 were used as the material for the study. The possibility of creating a regional forecast is due to the scale of field research (40 fields were surveyed in different areas of the Belgorod region). The reliability of the results is determined by the use of the field examination method tested in long-term practice. The scientific justification of the stability of the growth of the identified species of weeds in the Belgorod region ensures the development of a regional forecast for a long-time perspective. Thirty-two species of weed plants were recorded, among which annual species predominate (65.63%). The phytosanitary role of each species was revealed by determining the activity of each in the cenoflor of agrophytocenoses. A group of highly active weed species include barnyard millet, lamb's-quarters, yellow foxtail, black nightshade, sow thistles, wild buckwheat, redroot. Rather active species include: corn bindweed, milk thistle, black bindweed. The group of little active includes 13 species: sagebrush, common cocklebur, rocket cress, hedge-nettle betony, tansy mustard, bird vetch, mallow, forking larkspur, sumpfweed, prickly lettuce, common arache, water pepper, wild chamomile. Nine inactive species were identified and occasionally recorded in corn crops. The distribution of species composition by groups of different activity status will be preserved in a long time period, provided that the state of hydrothermal conditions is unchanged, the structure of sown areas is preserved, and the peculiarities of regional technology of maize cultivation and the system of protective measures are observed.

Keywords: monitoring, species activity, multi-year regional forecast

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INTRODUCTION

Corn (*Zea mays* L.) is a widely grown forage crop in many countries around the world, also grown for grain. In the Russian Federation, corn crops for forage occupy 1,261.4 thousand hectares, of which 400.1 thousand hectares are in the Central Federal District (CFD), and 41.2 thousand hectares are in the Belgorod Region, which is part of this district. Fully ripe corn crops for grain occupy much larger areas: 1,845.2 thousand hectares in the Russian Federation, 850.6 thousand hectares in the CFD, and 96.2 thousand hectares in the Belgorod Region[1].

Harmful objects in agricultural crops reduce the yield by more than 35%, with 9.5% of losses due to weeds [1]. The problem of weed infestation of corn crops, like any cultivated crop, is relevant both in Russia and abroad [2–6]. In the Russian Federation, corn crop losses due to

[1]Cultivated areas of the Russian Federation in 2023 // Federal State Statistics Service (Rosstat). https://rosstat.gov.ru/storage/mediabank/posev-4cx_2023.xlsx (date of reference 20 February 2024).

weeds average about 25%[2]. In 2021 and 2022, the area of weed infestation of corn crops in the Russian Federation exceeded 2,000 thousand hectares, in 2023 it decreased to 1,311.3 thousand hectares, but in 2024 it increased again to 1,611.59 thousand hectares[3]. It is indicated that the numerical composition of biological groups of weeds representing the destructive part of agrophytocenoses in corn crops is not the same in different federal districts on the territory of the European part of the Russian Federation (see footnote 3) (see Table 1).

These data are important for tracking the development trends of segetal flora in the regions and the characteristics of the formation of the types of weed infestation that occur when species from different biological groups are combined, but are less suitable for plant protection practice, since the same group in different regions may include different types of weeds that require different herbicide solutions [7].

Analysis of scientific publications shows differences in the composition of commonly encountered weed species in corn crops grown in different regions of the Russian Federation. Such species as lamb's-quarters (*Chenopodium album* L.), barnyard grass (*Echinochloa crus-galli* (L.) Beauv.), green amaranth (*Amaranthus retroflexus* L.), green bristlegrass and yellow foxtail (*Setaria viridis* (L.) Beauv. s. l., *Setaria pumila* (Poir.) Roem. et Schult.) are the dominant weeds in corn crops in the southern regions of the European part of the Russian Federation[4] (see footnote 2) [8, 9], in the irrigated zone of the Volga Delta [10], in the middle part of the Central Federal District [1, 12], in the Lower Volga region [13], in the south of the Non-Black Earth Zone [14]. In the southern regions, the species composition of weeds is replenished with common ragweed (*Ambrosia artemisiifolia* L.), common cocklebur (*Xanthium strumarium* L.) and China jute (*Abutilon theophrastii* Medik.) (see footnote 3) [9], moreover, common ragweed is not indicated for the Volga Delta [9]. In addition to these species, corn crops in Kabardino-Balkaria are contaminated with Johnson

Табл. 1. Преобладающие биологические группы сорных растений в отдельных федеральных округах, шт./м²

Table 1. Predominant biological groups of weed plants in individual federal districts, pcs./m²

Group of weeds	Federal District			
	Central	Southern	North Caucasian	Volga
Spring: early	4,4	2,1	3,6	2,7
late	8,3	3,2	6,7	5,4
Dormant	0,5	0,1	0,7	0,2
Biennial	0,3	–	0,1	–
Taproot plants	0,02	–	0,01	0,05
Rootstock plants	0,3	–	1,6	0,3
Root-sucker plants	1,9	0,2	2,9	1,9

[2]*Nakaev S.M.A., Okazova Z.P.* Dominant weeds and their harmfulness in corn crops // Modern Science Success, 2017, vol. 2, N 12, pp. 199–201.

[3]Review of the phytosanitary condition of agricultural crops in the Russian Federation in 2023 and a forecast for the development of harmful objects in 2024. Moscow, 2024. 1281 p.

[4]*Mysnik E.N., Zakota T.Yu.* The structure of the species composition of weeds in corn crops in the steppe zone of the Krasnodar Territory // Bulletin of Plant Protection. 2018. N 4 (98). pp. 50–53.

grass (*Sorghum halepense* (L.) Pers.), couch grass (*Cynodon dactylon* (L.) Pers.), arched winter cress (*Barbarea arcuata* (Opiz ex J. et C Presl) Reichb.) and common horsetail (*Equisetum arvense* L.) [8]. Black nightshade is often found in corn crops in the Volga Delta (*Solanum nigrum* L.), trailing hollyhock (*Hibiscus trionum* L.), mountain bluet (*Acroptilon repens* (L.) DC.), corn bindweed (*Convolvulus arvensis* L.) and couch grass (*Elytrigia repens* (L.) Nevski) [10]. In the middle part of the Central Federal District, in addition to species that contaminate corn crops in all regions of its cultivation in the European part of the Russian Federation, there are species such as common spurrey (*Spergula arvensis* L.), corn gromwell (*Lithospermum arvense* L.), shepherd's purse (*Capsella bursa-pastoris* (L.) Medik.), German camomile (*Tripleurospermum inodorum* (L.) Sch. Bip.), field pansy (*Viola arvensis* Murray) [11], as well as cleavers (*Galium aparine* L.), common fumitory (*Fumaria officinalis* L.), field pennycress (*Thlaspi arvense* L.) [12]. Corn bindweed and field milk thistle are also registered here (*Sonchus arvensis* L.), grey thistle (*Cirsium incanum* (S.G. Gmel.) Fisch.) [12] is also often found in corn crops in the Lower Volga region and in the south of the Non-Black Earth Zone [12–14].

In the south of the Non-Chernozem zone, common horsetail and pinweed are often found. (*Erodium cicutarium* (L.) L'Herit.), Canada fleabane (*Erigeron canadensis* L.), types of hemp-nettles (*Galeopsis* spp.), clown's woundwort (*Stachys palustris* L.) [14]. A similar picture, showing differences in the species composition of weeds that are problematic in crops of one crop grown in different regions, also characterizes winter wheat crops [15], which determines the relevance of regional studies.

In plant protection practice, the development of a set of protective measures in a separate agroecosystem is based on the results of monitoring the species composition and number of weeds in individual fields within its boundaries, and is therefore suitable for the fields of a given agroecosystem [16]. Conducting phytosanitary monitoring simultaneously in several farms in one region allows us to draw a conclusion about the contamination of all or individual crops in this region [17]. To characterize the phytosanitary situation of crops in a region or area, large-scale phytosanitary monitoring covering a large number of fields is necessary. An objective assessment of the phytosanitary situation in agrocenoses determines the forecast of the further presence of harmful objects in them. A phytosanitary forecast is a "probabilistic scientifically substantiated judgment on the dynamics of populations of harmful objects in the future, based on the patterns identified in the past" [18]. The subject of the forecast is the dynamics of not only the number, but also the species composition of the destructive part of agrophytocenoses, and the reliability of the forecast depends on the reliability and scale of the monitoring results. The regional forecast scientifically predicts the presence, at least in the next field season, of a complex of weeds identified in the current season. It is possible to give a forecast of the presence in the agrophytocenoses of the region for a period of a number of years only for those species of weeds whose stable growth in this region is scientifically substantiated.

Weeds are wild plants of secondary habitats [19], therefore the ranges of species, as well as regional species complexes, are formed in accordance with the level of heat and moisture supply of the territory and the level of requirements of each individual species for heat and moisture factors[5]. On this basis, an ecological-geographical analysis is carried out, which is used in studying the distribution of weeds[6]. Using this analysis, a complex of weeds was previously identified for which the territory of the Belgorod region is optimally suited in terms

[5]*Alekhine V.V., Kudryashov L.V., Govorukhin V.S.* Plant geography with the basics of botany. Moscow: Uchpedgiz, 1961, 532 p.

[6]*Gillham J.H., Hild A., Johnson J.H., Hunt E.R. Jr., Whitson T.D.* Weed invasion susceptibility prediction (WISP) model for use with geographic information systems. Arid land research and management. 2004. Vol. 18. Is. 1. P. 1–12. DOI: 10.1080/15324980490244933.

of heat and moisture supply[7]. The complex of weeds that forms agrophytocenoses in the crops of a separate crop represents cenoflora as a division of segetal flora and, like all weed flora, can be predicted for the territory of the Belgorod region in a significant time perspective [15, 19].

The purpose of the study is to identify the species composition of weeds in corn crops in the Belgorod region and to develop a long-term forecast of their growth in these agrophytocenoses.

The research objectives are:

– to monitor corn crops;

– to systematize the data, establish the species composition of weeds;

– to determine the degree of activity of species in crops.

MATERIAL AND METHODS

The object of the study is the species composition of weeds in agrophytocenoses of corn crops in the Belgorod region. The material for the study was the data of phytosanitary monitoring of the state of corn crops in the Belgorod region in 2020, 2021. Monitoring (40 fields were surveyed) was carried out in accordance with the methodology of geobotanical survey of crops in relation to weeds[8].

The materials are systematized using a specialized database "Weeds of the fields of the Russian Federation"[9]. The taxonomic structure of the species composition was established by the method of floristic analysis[10]. The names of taxa are given in accordance with the floristic summary compiled for plant protection practitioners[11].

The phytosanitary role of weed species in corn crops was revealed by determining their activity in agrophytocenoses using the method of T.A. Palkina[12]. According to the methodology, the activity of a species in the formation of agrocenoses in crops of one culture (partial) was determined taking into account two indicators: the constancy of the occurrence of this species in the crops of a given culture and the average projective cover in the cenoflora.

Six classes of occurrence constancy were identified: 1st class: the species was found in less than 10% of fields, 2nd – in 10–20, 3rd – in 21–40, 4th – in 41–60, 5th – in 61–80, 6th – in 81–100% of fields.

Abundance classes were identified based on the projective cover of the species in the communities: 1st class – single plants, 2nd – abundance less than 0.5%; 3rd – abundance 0.5–1.0, 4th – abundance 1.1–2.0, 5th – abundance 2.1–5.0, 6th – abundance more than 5%. Based on the combination of these indicators, weed species were divided into 6 categories (1 – particularly active, 2 – highly active, 3 – moderately active, 4 – fairly active, 5 – slightly active, 6 – inactive) in accordance with the scale given in Table 2.

RESULTS AND DISCUSSION

Low foxtail and unidentified foxtail species have been recorded in corn crops. In the plant protection system, closely related species that require the same control measures are often grouped together, such as "vetch species", "hemp nettle species" or "foxtail *Setaria* L." Therefore, these species were combined for analysis into a group of "foxtail species" and

[7]*Luneva N.N., Fedorova Yu.A.* Ecological and geographical substantiation of the formation of a species complex of weeds in the Belgorod region // Biological species in the structural and functional hierarchy of the biosphere: Proc. XV int. scientific and practical. ecological conf. (Belgorod, October 8-12, 2018). Belgorod: National Research University "BelSU", 2018, pp. 104-108.

[8]*Luneva N.N.* Technological methods of accounting and monitoring of weeds in agroecosystems // High-performance and high-precision technologies and methods of phytosanitary monitoring. St. Petersburg: All-Russian Research Institute of Plant Protection, 2009, pp. 39–56.

[9]*Mysnik E.N., Luneva N.N., Sokolova T.D., Nadtochiy I.N.* Weeds of the fields of the Russian Federation. Database registration certificate No. 2021522847 dated 09.12. 2021.

[10]*Tolmachev A.I.* Methods of comparative floristics and problems of florogenesis. Novosibirsk: Nauka, 1986, 195 p.

[11]*Luneva N.N., Mysnik E.N.* Modern botanical nomenclature of weed species of the Russian Federation // Supplements to the journal " Plant Protection News", 2018. Vol. 26. 80 p. DOI: 10.5281/zenodo.1241599.

[12]*Palkina T.A.* Structure of segetal flora of Ryazan region // Herald of Ryazan state agrotechnological university named after P. A. Kostychev, 2015, N 27 (3), pp. 26–32.

Табл. 2. Баллы парциальной активности видов растений сегетальной флоры в агрофитоценозах культуры (см. сноску 12)

Table 2. Partial activity scores of plant species of segetal flora in agrophytocenoses of the culture (see footnote 12)

Abundance score	Projective cover, %	Activity score in the persistency class					
		VI	V	IV	III	II	I
		81–100	61–80	41–60	21–40	10–20	< 10
6	> 5	1	1	2	3	4	5
5	2,1–5,0	2	2	3	4	4	5
4	1,1–2,0	2	2	3	4	4	5
3	0,5–1,0	3	3	4	5	5	5
2	< 0,5	4	4	4	5	5	6
1	Sporadically	5	5	5	5	6	6

considered as one species.

As a result of processing the data obtained during the survey of corn fields in the Belgorod region, 32 species of weeds from 30 genera and 17 families were identified, which is indicated in Table 3.

The presented systematic spectrum, the leading families of which are Compositae, Cruciferae, Polygonaceae, Gramineae and Chenopodiaceae, differs from the composition of the leading families of the taxonomic spectrum of agrophytocenoses of winter wheat crops in the Belgorod region by the presence of the Chenopodiaceae family instead of the Leguminosae family [15]. This indicates both the unity of the segetal flora of the Belgorod region and the differences between its constituent coenofloras. The majority (70.59%) of the families are represented by one genus and one species. Taxonomic diversity is expressed by the following average indicators: the number of species in a family is 1.88, the number of genera in one family is 1.76, the number of species in one genus is 1,07.

Phytocenotic activity, showing the phytosanitary role of each type of weed in agrophytocenoses of corn crops, is presented in Table 4.

Analysis of the identified species by lifespan showed that annual weed species predominate (65.63% are annual and biennial species). The

Табл. 3. Структура сорного компонента в агрофитоценозах посевов кукурузы в Белгородской области (2020, 2021 гг.)

Table 3. Structure of weed component in agrophytocenoses of corn crops (Belgorod region, 2020, 2021)

Weed plant family	Number in the family	
	types	genera
Composite plants (Compositae Giseke)	11	8
Cruciferous plants (Cruciferae Juss.)	4	4
Buckwheat plants (Polygonaceae Juss.)	3	3
Gramineous plants (Gramineae Juss.)	2	2
Goosefoot family (Chenopodiaceae Vent.)	2	2
Bindweed family (Convolvulaceae Juss.)	1	1
Legumes (Leguminosae Juss.)	1	1
Amaranthaceous (Amaranthaceae Juss.)	1	1
Poppy family (Papaveraceae Juss. (incl. Fumariaceae DC.))	1	1
Parsley family (Umbelliferae Juss.)	1	1
Heliotrope family (Boraginaceae Juss. (incl. Hydrophyllaceae R. Br.))	1	1
Equisetaceous plants (Equisetaceae Michx. ex DC.)	1	1
Mallow family (Malvaceae Juss.)	1	1
Labiate family (Labiatae Juss.)	1	1
Figwort family (Scrophulariaceae Juss. s. l. (incl. Orobanchaceae Vent.))	1	1
Nightshade family (Solanaceae Juss.)	1	1
Crowfoot family (Ranunculaceae Juss.)	1	1

perennial group includes 10 species: corn bindweed, milk thistle, sagebrush, gray sow thistle, field falcaria, common wormwood, arched winter cress, common horsetail, bird vetch, common toadflax, small mallow.

No particularly active weed species were identified in corn crops. The group of highly active species in corn crops consisted of seven weed species. Barnyard grass, lamb's-quarters and foxtail species had high (95.00–100.00%) rates of occurrence and projective cover (3.81–4.69%) in the surveyed fields. Black nightshade, grey sow thistle, wild buckwheat and pigweed had slightly lower incidence and projective cover rates: 65.00–80.00 and 1.32–3.44%, respectively. Moderately active weed species were

Табл. 4. Виды сорных растений и статус их активности в посевах кукурузы в Белгородской области (2020, 2021 гг.)
Table 4. Weed plant species and their activity status in corn crops (Belgorod region, 2020, 2021)

Type of weed plants	Occurrence, %	Occurrence constancy class	Projective cover	Abundance class	Activity category	Activity status
Foxtail types (*Setaria* spp.)	100,00	VI	4,69	5	2	HA
Lamb's quarters (*Chenopodium album* L.)	97,50	VI	3,81	5	2	HA
Barnyard grass (*Echinochloa crusgalli* (L.) Beauv.)	95,00	VI	3,84	5	2	HA
Green amaranth (*Amaranthus retroflexus* L.)	80,00	V	3,44	5	2	HA
Black bindweed (*Fallopia convolvulus* (L.) A. Löve)	75,00	V	1,32	4	2	HA
White-felted thistle (*Cirsium incanum* (S.G. Gmel.) Fisch.)	67,50	V	1,22	4	2	HA
Black nightshade (*Solanum nigrum* L.)	65,00	V	3,14	5	2	HA
Hedge-nettle betony (*Stachys annua* (L.) L.)	35,00	III	0,22	2	5	LA
Tansy mustard (*Descurainia sophia* (L.) Webb ex Prantl)	35,00	III	0,10	2	5	LA
Corn bindweed (*Convolvulus arvensis* L.)	32,50	III	1,49	4	4	QA
Arched winter cress (*Barbarea arcuata* (Opiz ex J. et C Presl) Reichb.)	30,00	III	0,78	3	5	LA
Common cocklebur (*Xanthium strumarium* L.)	30,00	III	0,73	3	5	LA
Sagebrush (*Artemisia vulgaris* L.)	30,00	III	0,10	2	5	LA
Sumpfweed (*Cyclachaena xanthiifolia* (Nutt.) Fresen.)	17,50	II	1,07	3	5	LA
Forking larkspur (*Consolida regalis* S.F. Gray)	17,50	II	0,10	2	5	LA
Field milk thistle (*Sonchus arvensis* L.)	15,00	II	1,20	4	4	QA
Little mallow (*Malva pusilla* Smith.)	15,00	II	0,10	2	5	LA
Knotgrass (*Polygonum aviculare* L. s. str.)	10,59	II	9,20	6	4	QA
bird vetch (*Vicia cracca* L.)	10,00	II	0,06	2	5	LA
German camomile (*Tripleurospermum inodorum* (L.) Sch. Bip.)	7,50	I	1,50	4	5	LA
Field falcaria (*Falcaria vulgaris* Bernh.)	7,50	I	0,10	2	6	NA
Water pepper (*Persicaria hydropiper* (L.) Delarbre)	5,00	I	7,80	6	5	LA
Common orache (*Atriplex patula* Bouscher ex DC.)	5,00	I	0,98	3	5	LA
Prickly lettuce (*Lactuca serriola* L.)	5,00	I	0,50	3	5	LA
Spiny annual sow thistle (*Sonchus asper* (L.) Hill.)	5,00	I	0,12	2	6	NA
Common fumitory (*Fumaria officinalis* L.)	5,00	I	0,10	2	6	NA
Common horsetail (*Equisetum arvense* L.)	5,00	I	0,01	2	6	NA
Field pennycress (*Thlaspi arvense* L.)	2,50	I	0,10	2	6	NA
Common wormwood (*Artemisia absinthium* L.)	2,50	I	0,10	2	6	NA
Shepherd's purse (*Capsella bursa-pastoris* (L.) Medik.)	2,50	I	0,10	2	6	NA
Field scorpion grass (*Myosotis arvensis* (L.) Hill)	2,50	I	0,10	2	6	NA
Common toadflax (*Linaria vulgaris* Mill.)	2,50	I	0,10	2	6	NA

Примечание. ОА – особоактивные виды, ВА – высокоактивные, СА – среднеактивные, ДА – довольноактивные, МА – малоактивные, НА – неактивные виды.

not identified in corn crops. The group of fairly active species consisted of three species: corn bindweed, field sow thistle and bird's knotweed. Corn bindweed has a higher occurrence rate (32.50%), and bird's knotweed has a projective cover (9,20%).

The group of low-active species in corn crops consisted of 13 species of weeds. Common wormwood, common cocklebur, arched winter cress, hedge-nettle betony and Sofia tansy mustard had a frequency of occurrence in the surveyed fields of 30.00–35.00% with a projective cover of 0,10–0,78%. Bird vetch, small mallow, forking larkspur, sumpfweed had a lower occurrence (10.00–17.50%) with a projective cover of 0.06–1.07%. Prickly lettuce, common orache, water pepper and wild chamomile were found in an even smaller number of fields (5.00–7.50%) with a projective cover of 0,50–7,80%.

The group of inactive species in corn crops consisted of nine weed species: field falcaria, rough sow thistle, common fumitory, common horsetail, field pennycress, common wormwood, shepherd's purse, field forget-me-not and common toadflax. In the surveyed fields, they had low rates of occurrence and projective cover: 2.50–7.50 and 0.01–0.12%, respectively.

For 29 out of 32 weed species infesting corn crops in the Belgorod Region, this territory is suitable for growth in terms of heat and moisture supply (see footnote 7). It is not possible to prove the suitability of the survey territory for the growth of three more weed species identified in corn crops (common wormwood, common orach, small mallow) using the method of eco-geographical analysis due to the lack of electronic maps of their distribution. However, the regular occurrence of these species during monitoring allows them to be included in the composition of the cenoflora of corn crops in the Belgorod Region. Not all of the identified species have high phytosanitary significance; the most widespread and abundant in the agrophytocenoses of this crop are the seven highly active and three fairly active species named above.

CONCLUSION

1. The identified composition of the cenoflora of agrophytocenoses of corn crops in the Belgorod region is stable and predictable in the long term (long-term regional forecast). The species composition and distribution of species by groups of different activity status will be preserved if the state of hydrothermal conditions in the region does not change and the structure of crop areas is preserved, and regional agrotechnology for corn cultivation and a system of protective measures are applied.

2. The results were obtained based on the survey of a large number of fields; therefore, they are of an average nature, i.e. not all identified species are present and will continue to grow in all fields under corn crops. This significantly increases the role of phytosanitary monitoring at the level of individual agroecosystems to identify which of the species predicted in agrophytocenoses of corn crops grow in a given farm, and to promptly track the introduction of species from other regions.

3. The obtained results are important for developing a long-term regional forecast of the growth of the identified complex of weeds in corn crops in the Belgorod region, on the basis of which it is possible to adjust the regional system of protective measures in this crop. Knowledge of the species composition of high phytosanitary significance is important for planning the purchase of means of protecting corn crops from weeds on a regional scale. Knowledge of the species composition of the entire complex of weeds in corn crops is important for tracking the changes that may occur when the structure of crop areas and the melioration system are disrupted.

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Воздействие Дивиденда Экстрим, КС и его композиции с Новохизолем на фитосанитарное состояние семян и посевов яровой мягкой пшеницы

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В 2020 и 2021 гг. проведено исследование по изучению влияния протравителя Дивиденд Экстрим, КС и его композиции с Новохизолем на зараженность семенного материала и фитосанитарную ситуацию в посевах яровой мягкой пшеницы сорта Новосибирская 31, выращиваемой на безотвально-обработанном выщелоченном черноземе. Опыт включал три варианта обработки семян: 1) контроль (без обработки); 2) коммерческий фунгицид Дивиденд Экстрим, КС (дифеноконазол, 92 г/л + мефеноксам, 23 г/л); 3) композиция, содержащая уменьшенное в 13,4 раза количество действующих веществ Дивиденда Экстрим, КС и Новохизоль (дифеноконазол, 6,88 г/л + мефеноксам, 1,71 г/л + Новохизоль, 2,5% + янтарная кислота, 1,25%; формуляция предложена Новосибирским институтом органической химии им. Н.Н. Ворожцова СО РАН). Установлена высокая биологическая эффективность (93,3 и 100,0%) обработки семян Дивидендом Экстрим, КС (расход 0,5–0,6 л/т) против *Bipolaris sorokiniana* (Sacc.) Shoemaker (syn. *Helminthosporium sativum* Pam., King et Bakke; *Helminthosporium sorokinianum* Sacc.). Сниженная норма расхода ослабляла эффект защиты вдвое. Биологическая эффективность композиции с Новохизолем уступала коммерческому протравителю как с пониженной, так и с повышенной нормой расхода. Оба препарата снижали численность спор *B. sorokiniana* в почве, но на вегетативном этапе развития растений уменьшение их плотности отмечали при обработке семян Дивидендом Экстрим, КС. В фазе молочной спелости более низкие показатели развития обыкновенной корневой гнили фиксировали в обоих опытных вариантах при большей эффективности коммерческого фунгицида. Выявлено, что защита семян фунгицидной композицией с Новохизолем может опосредованно (через ростостимуляцию растения-хозяина) влиять на пораженность листьев как септориозом, так и бурой ржавчиной, увеличивая развитие данных болезней в 2,1 и 2,5 раза соответственно.

Ключевые слова: Дивиденд Экстрим, КС, Новохизоль, яровая мягкая пшеница, развитие и распространенность болезни

Impact of the Dividend Extreme, SC and its composition with Novochizol on the phytosanitary condition of seeds and crops of spring soft wheat

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In 2020 and 2021, a study was conducted to investigate the effect of the dressing agent Dividend Extreme, SC and its composition with Novochizol on seed infestation and phytosanitary situation in the crops of spring soft wheat Novosibirskaya 31 cultivated on no-till leached chernozem. The experiment included 3 seed treatment options: 1) control (without treatment); 2) commercial fungicide Dividend Extreme, SC (Difenoconazole, 92 g/l + Mefenoxam, 23 g/l); 3) composition with a 13.4-fold reduced amount of active ingredients Dividend, Extreme, SC and Novochizol (Difenoconazole, 6.88 g/l + Mefenoxam, 1.71 g/l + Novochizol, 2.5% + succinic acid, 1.25%; the formulation was proposed by the N.N. Vorozhtsov Novosibirsk Institute of Organic Chemistry SB RAS). High biological efficiency (93.3 and 100%) of seed treatment with Dividend Extreme, SC (consumption 0.5–0.6 l/t) against *Bipolaris sorokiniana* (Sacc.) Shoemaker (syn. *Helminthosporium sativum* Pam., King et Bakke; *Helminthosporium sorokinianum* Sacc.) was established. The reduced application rate weakened the protection effect by half. The biological efficiency of the composition with Novochizol was inferior to the commercial seed treatment with both reduced and increased application rates. Both preparations reduced the number of *B. sorokiniana* spores in the soil, but at the vegetative stage of plant development, their lower density was noted when treating seeds with Dividend Extreme, SC. In the milk ripeness phase, lower development of common root rot was recorded in both experimental variants with a predominance of efficiency from treatment with a commercial fungicide. It was found that protection of seeds with the fungicide composition Novochizol can indirectly (through growth stimulation of the host plant) affect the infestation of the leaves with both septoriose and brown rust, increasing the development of these diseases by 2.1 and 2.5 times.

Keywords: Dividend Extreme, SC, Novochizol, spring soft wheat, development and prevalence of the disease

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INTRODUCTION

The phytosanitary condition of agroecosystems is one of the indicators affecting their productivity [1, 2]. The effectiveness of the fight against phytopathogens and the nature of the impact on the host plant are determined by the active ingredient (a.i.) of the pesticide, its formulation[1] and the application rate[2]. In order to increase the level of protection of grain crops, combined preparations are being developed. In recent years, the number of such preparations has increased almost 2 times.

To control the formation of root rot of various etiologies, the two-component systemic fungicide Dividend Extreme (OOO Syngenta, Russia) is recommended [3, 4]. The formulation is a suspension concentrate, the active ingredients are difenoconazole, 92 g/l + mefenoxam, 23 g/l. Difenoconazole, a triazole derivative, belongs to the largest group of systemic fungicides that inhibit the synthesis of sterols. It has specific activity against smut diseases, root rots and seed mold. Due to moderate systemicity, it gradually penetrates the xylem of the plant into

[1]*Karakotov S.D., Popov S.Ya., Dymov Yu.A.* Influence of the preparative form of the seed dressing on its biological efficiency // Zemledelie, 2016, N 3, pp. 44–47.

[2]*Dymov Yu.A.* Research of biological efficiency of some promising seed treatments against root rots on spring wheat // Vestnik Orel GAU, 2016, N 4 (61), pp. 84–90.

new growth, completely inhibits the growth of subcuticular mycelium, and reduces the level of sporulation of the pathogen. It practically does not move in the soil[3]. According to the manufacturer, difenoconazole is the most effective active ingredient for controlling dark-colored fungi[4]. It is reported about the proven high efficiency of difenoconazole and mefenoxam in the fight against such diseases of grain crops as alternaria, helminthosporium and pythium root rot. The high concentration of difenoconazole in Dividend Extreme, SC allows to reliably protect seedlings, the root system and the tillering node from helminthosporium root rot for a period of up to 30 days. The drug does not have a side retardant effect on seedlings and eliminates their spareness.

Mefenoxam (R-isomer of metalaxyl) is a phenylamide, a systemic fungicide with protective, eradicating and curative action. Mefenoxam inhibits the formation of proteins in fungi, suppresses the synthesis of ribosomal RNA, mycelial growth, the formation of zoospores, oospores and chlamydospores, but has virtually no effect on the germination of zoospores. Growth inhibition occurs only after the penetration of the parasite's haustoria into the plant cell[5]. Mefenoxam-based preparations are used to combat Pythium, Helminthosporium, Fusarium root rot, Alternaria seed infection and seed mold. Mefenoxam has a systemic effect at very low dosages (see footnote 3). During dressing, most of the fungicide accumulates in the seed coat, embryo and peripheral part of the endosperm. During germination of treated seeds, a significant part of the preparation is found in the cotyledons, and a smaller part in the stems and roots of the seedling. The period of protective action of preparations containing mefenoxam is 10–14 days; in the soil, the substance is active for 40-70 days (see footnote 5).

There are data on the activity of Dividend Supreme, SC (difenoconazole, 39.92 g/l + mefenoxam, 3.08 g/l + thiamethoxam, 92.30 g/l) against fusarium- helminthosporium (Leningrad Region) and helminthosporium -fusarium root rot of spring wheat (Saratov and Volgograd Regions). In the first case, the fungicide efficiency was 86.0–91.5%, in the second – 55.1–74.1%. When applied to winter wheat crops in the Krasnodar Region, 100% control of fusarium rot was recorded[6]. Due to the fact that many types of fungi that cause root rot are resistant to fungicides, continuous screening of modern preparations is carried out. Thus, against an artificial infectious background, high efficiency of Dividend Star, SC (difenoconazole, 30.0 g / l + cyproconazole, 6.3 g / l) against root rot pathogens *Bipolaris sorokiniana* and *Fusarium equiseti* (100.0 and 99.6%, respectively) was established [5].

There are reports that the combined use of seed dressings with anti-stress preparations enhances the host plant's defense mechanisms, reduces the negative effects of fungicides, and helps increase the yield of grain crops[7]. In particular, the tank mixture Dividend Star, 0.5 l/t + Fitosporin-M, 1.0 l/t, reducing the development of root rot by 2 times, increased the yield by 1.3–2.4 c/ha. This technique is considered one of the methods for greening the protection of spring wheat [6].

The polysaccharide chitosan is used as a biofungicide, the fungistatic action of which depends on its physicochemical properties, the type of microorganisms, the composition and concentration of chitosan-containing substances [7]. High fungistatic activity of chitosan in combination with succinic acid has been noted on fruit [8]. The combined effect of chitosan with

[3]*Belitskaya M.N., Gribust I.R., Baibakova E.V., Nefedyeva E.E., Shaikhiev I.G.* Research and comparative analysis of active substances of modern seed treatment agents for grain crops // Herald of Technological University, 2015, vol. 18, N 9, pp. 32–36.

[4]www.syngenta.ru

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[6]*Grishechkina L.D., Burkova L.A., Dolzhenko V.I., Silaev A.I., Milyutenkova T.I.* New insectofungicide combination Dividend Supreme for wheat protection // Zemledelie, 2016, N 1, pp. 41–44.

[7]*Lukhmenev V.P.* Greening the protection of spring wheat through the use of biological preparations and growth regulators in tank mixtures with pesticides // Izvestia Orenburg State Agrarian University, 2004, N 4 (4), pp. 16–17.

vanillin and salicylic acid on the resistance of wheat to brown rust (pathogen *Puccinia recondita*) and dark brown spot (pathogen *Cochliobolus sativus*) has been established: resistance to both pathogens is increased by chitosan, modified in the first case with salicylic acid, in the second - with vanillin. The addition of chitosan salicylate to the medium for submerged cultivation of *Bacillus subtilis* strains VKM B-2604D and VKM B 2605D and to the Vitaplan preparation increased its protective effect in the wheat – *C. sativus* pathosystem by 1.5–2.0 times [9]. It is assumed that the increased biological activity of the new forms of the Vitaplan preparation is the result of a combination of the fungicidal activity of the biopreparation and the induction of plant defense reactions by chitosan salicylate [10].

Chitosan salicylate is also capable of controlling *Phytophthora infestans* (Mont.) de Bary[8]. In field conditions, high biological efficiency (93.1; 88.0 and 80.0%) of a chitosan-tebuconazole composition against *P. recondita*, *Septoria nodorum* and *Blumeria graminis* was demonstrated with a single treatment at the beginning of earing of soft spring wheat plants. The addition of chitosan salicylate reduced the fungicide consumption by 5.5 times and increased grain yield by 0.55 t/ha [11]. The obtained results indicate the possibility of using chitosan to create fungicide compositions in more advanced forms – with low consumption rates while maintaining equivalent efficiency [12, 13].

The purpose of this study is to investigate the effect of the two-component fungicide-dressing agent Dividend Extreme, SC and its composition with Novochizol, containing a reduced amount of difenoconazole and mefenoxam, on the phytosanitary condition of seeds and crops of spring soft wheat grown after fallow on non-moldboard-cultivated unfertilized leached chernozem in the northern forest-steppe of the Novosibirsk region.

MATERIAL AND METHODS

The study was conducted in 2021 and 2022 on the experimental field of the Siberian Federal Scientific Centre of Agro-BioTechnologies of the Russian Academy of Sciences, located in the central forest-steppe Priobsky agrolandscape region in the Novosibirsk region. The soil is leached medium loamy chernozem of medium thickness. The main soil cultivation is deep non-moldboard loosening with SibIME tines, in the spring – moisture closing with BIG-3A harrows and pre-sowing cultivation (6–8 cm) diagonally of the main cultivation. The forecrop is fallow land without fertilization. Spring wheat of the Novosibirskaya 31 variety was used in the experiment. Sowing was carried out on May 20 (2021) and 21 (2022) with an SZS-2.1 seeder (with anchor coulters). The seeding rate is 6 million grains/ha. Harvesting was carried out on August 26 (2021) and September 1 (2022). Seed treatment was carried out with moistening (10 l H2O/t) 6 days before sowing in closed plastic bags.

The experiment included three seed treatment options:

1) control (without treatment);

2) commercial fungicide Dividend Extreme, SC (difenoconazole, 92.0 g/l + mefenoxam, 23.0 g/l);

3) Dividend Extreme, SC (the amount of active ingredient is reduced by 13.4 times: difenoconazole – 6.88 g/l, mefenoxam – 1.71 g/l) + Novochizol (2.5%) + succinic acid (1.25%). The formulation was proposed by the Novosibirsk Institute of Organic Chemistry, SB RAS.

An aqueous suspension of Novochizol (provided by Novochizol SA, Switzerland[9]) was prepared by dissolving succinic acid (500 mg/100 ml of sterile water) and gradually adding Novochizol (1000 mg/100 ml of succinic acid solution). To intensify the process, an ultrasonic technological device UZTA-0.4/22-OM (U sonic, Russia[10]) was used at maximum power for

[8] *Vasyukova N.I., Ozeretskovskaya O.L., Chalenko G.I., Gerasimova N.G., Lvova A.A., Ilyina A.V., Levov A.N., Varlamov V.P., Tarchevsky I.A.* Immunomodulatory activity of chitosan derivatives with salicylic acid and its fragments // Applied Biochemistry and Microbiology, 2010, vol. 46, N 3, pp. 379–384.

[9] www.novochizol.ch

[10] www.u-sonic.ru

1 hour. To compensate for evaporation caused by prolonged ultrasonic treatment, sterile water was added. To sterilize the solution, filtration was performed using apyrogenic acetate cellulose filters (pore size 0.45 μm; Sartorius, Germany). The preparation was stored as a 1% stock solution at 4 °C and used within 1 week. Dilution was performed immediately before seed treatment [13].

The experiment was repeated three times. The plot area was 14.7 m^2. In the tillering phase, to limit the growth of weeds, continuous treatment was carried out with a tank mixture of herbicides Axial, EC, 1.0 l/ha + Primadonna, SE, 0.4 l/ha + Hextar, WDG, 10.0 g/ha. The efficiency of the fungicide-dressing agent Dividend Extreme, SC and its composition with Novochizol was studied in dynamics: phases of two, four leaves and milky ripeness. The main evaluation indicators were the development index (R, %) and the disease prevalence index (*P*, %) with the conversion of the point score into a percentage[11]. To analyze the damage of the root system to common root rot, ten plants were selected at ten points of the plot (n = 100). In the upper root layer of the soil, the intensity of accumulation of spores of the fungus *Bipolaris sorokiniana* Shoem., which is the main causative agent of the disease, was assessed (conidia / 1 g of air-dry soil; average sample – 1000 g from ten points). Sampling was carried out by shaking the soil off the plants[12, 13].

The study was conducted in the years with contrasting degrees of humidity. In 2021, 187.9 mm of precipitation fell from May to August (24,8; 73,3; 22,4; 67,4 mm), in 2022 – 113,1 mm (2,5; 59,0; 28,4; 23,2 mm). Relative to the long-term average, the difference by months was: –45,2, +64,3, –68,9, +2,1 in 2021, –93,1, +1,7, –60,6, –64,8 in 2022. The average daily temperature in May – August 2021 reached 15,1; 17,2; 21,0; 19,1 °C (deviation from the long-term average: +4,0, –0,5, +0,7,+2,8 °C), during the same period 2022 – 15,3; 17,2; 18,9; 16,5 °C (+5,0, +0,5, –0,1, +0,7 °C). According to the accepted classification, in terms of moisture levels, 2021 can be classified as moderately deficient, 2022 as extremely dry[14].

RESULTS AND DISCUSSION

Two-year field trials showed that during the vulnerable growth period (seedlings – two leaves), the use of the commercial fungicide Dividend Extreme, SC as a seed dressing reliably reduced the frequency of the plants affected by common root rot (by 1.8 and 2.0 times) (see Table 1). Biological efficiency by year differed slightly (52.2 and 58.1%). The development of the disease relative to the control indicators (R = 15.80 and 18.51%) decreased by 1.9 and 2.1 times. Thus, the positive effect of dressing with a commercial fungicide was more pronounced in the extremely dry growing season of 2022.

No annual decrease in the incidence of common root rot was observed in the Novochizol variant. Only in 2021 did the fungicidal effect of the experimental composition reach the level of the commercial product (R = 6.25%, biological efficiency – 60.40%) (see Table 1). In conditions of severe drought (2022), on the contrary, there was a tendency for the number of affected plants to increase. The damage to primary roots (R = 12.2%, $R_{control}$ = 16.0%) and, in particular, coleoptile (R = 11.2%, $R_{control}$ = 7.9%) was poorly controlled. For comparison, the biological efficiency of Dividend Extreme, SC this year reached 61.0 (primary roots) and 62.6% (coleoptile).

In the four-leaf phase, the dependence of the seed treatment efficiency on the wheat growing conditions was observed. In a more favorable season in terms of moisture, the level of damage to plants protected by a commercial fungicide

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Табл. 1. Динамика развития обыкновенной корневой гнили в посевах яровой пшеницы (полевой опыт), %
Table 1. Dynamics of common root rot development in spring wheat crops (field experiment), %

Indicator	Control		Dividend Extreme, SC, consumption 0,6 l/t		Dividend Extreme, SC + Novochizol + succinic acid, consumption 0,6 l/t		LSD_{05}	
	2021	2022	2021	2022	2021	2022	2021	2022
Two leaves stage								
Disease progress index	15,80	18,51	8,25	7,75	6,25	18,75	–	–
Disease prevalence	56,00	61,05	32,00	29,02	25,00	64,86	0,55	3,92
Four leaves stage								
Disease progress index	21,00	27,76	14,75	12,50	14,50	18,26	–	–
Disease prevalence	74,00	88,03	59,00	48,01	58,00	66,02	1,99	3,00
Milk-ripe stage								
Disease progress index	29,47	30,28	18,53	23,73	24,20	23,00	–	–
Disease prevalence	90,97	97,03	68,04	81,94	82,90	82,98	2,08	4,05

was 30.0% lower than in the control, in a dry season it was 55.0% lower. When treating seeds with a composition containing Novochizol, this dependence was not detected. In both years, its biological efficiency remained almost the same - 31.0 and 34.0%, respectively. A significantly lower prevalence of the disease than in the control was recorded in 2022: Dividend Extreme, SC reduced the proportion of diseased plants by 48.01%, Dividend Extreme, SC + Novochizol – by 66.02%. During the years of the study, the effectiveness of protecting the underground internode and the aboveground root part with the first treatment option reached 77.6 and 70.6%, with the second – 57.3 and 37.6%, i.e. 1.4 and 1.8 times less than the control ($R_{control}$ = 13,5 и 22,0%).

At the stage of milk ripeness, a decrease in the development of common root rot by 1.4 and 1.3 times, respectively, was observed when using both treatment options. At the same time, the commercial fungicide showed greater efficiency (R = 21.1% in 2021 and 2022). The prevalence of the disease in the control on average over the years of the study reached 94.0%, and in the treatment options it decreased by 1.2–1.3 times when using Dividend Extreme, SC, by 1.1 times – when seed dressing with a composition containing Novochizol.

In all three phases, soil samples from plant roots were studied using the flotation method used to determine the level of spore infestation by the main pathogen of common root rot. The results showed that at the early stage of organogenesis, the number of *B. sorokiniana* conidia in the root layer of plants protected by a commercial seed treatment agent decreased by 21.8% in the moderately humid season and increased by 18.2% in the extremely dry season (see Table 2). An annual decrease in spore density by 23.2–27.3% occurred under the influence of the experimental preparation with Novochizol.

On the subsequent date of recording (four-leaf stage), a smaller number of spores was found in the treated crops. As a result of treatment with Dividend Extreme, SC, the number of conidia decreased by 24.6–35.2%, after treatment with a composition with Novochizol – by 16.8–27.5% (control – 141.30 ± 3.75 and 113.80 ± 2.39 conidia/1 g of soil in 2021 and 2022). The same trend was maintained in the milk ripeness phase: due to the use of Dividend Extreme, SC,

Табл. 2. Динамика накопления спор *B. sorokiniana* в верхнем прикорневом слое (полевой опыт), конидий/1 г почвы*

Table 2. Dynamics of accumulation of *B. sorokiniana* spores in the upper root layer (field experiment), conidia/1 g soil*

Plant development stage	Year	Control	Dividend Extreme, SC, consumption 0,6 l/t		Dividend Extreme, SC + Novochizol + succinic acid, consumption 0,6 l/t	
			$M \pm m$	$t_{1\text{-}2,\ 05\ fact}$**	$M \pm m$	$t_{1\text{-}3,\ 05\ fact}$
Two leaves	2021	86,30 ± 3,15	67,50 ± 2,50	4,67	66,30 ± 1,25	5,90
	2022	55,00 ± 2,89	65,00 ± 2,04	2,83	40,00 ± 3,54	3,28
Four leaves	2021	141,30 ± 3,75	106,3 ± 2,39	7,87	117,50 ± 4,33	4,15
	2022	113,80 ± 2,39	73,80 ± 2,39	11,83	82,50 ± 1,44	11,22
Milk ripeness	2021	195,00 ± 5,40	141,30 ± 3,75	8,17	152,50 ± 4,33	6,14
	2022	1187,50 ± 12,67	796,20 ± 4,27	29,27	886,30 ± 12,97	16,61

*Economic threshold of harmfulness – 20 conidia/1 g soil; moderate population – 20–100 conidia/1 g; high – more than 100 conidia/1 g.

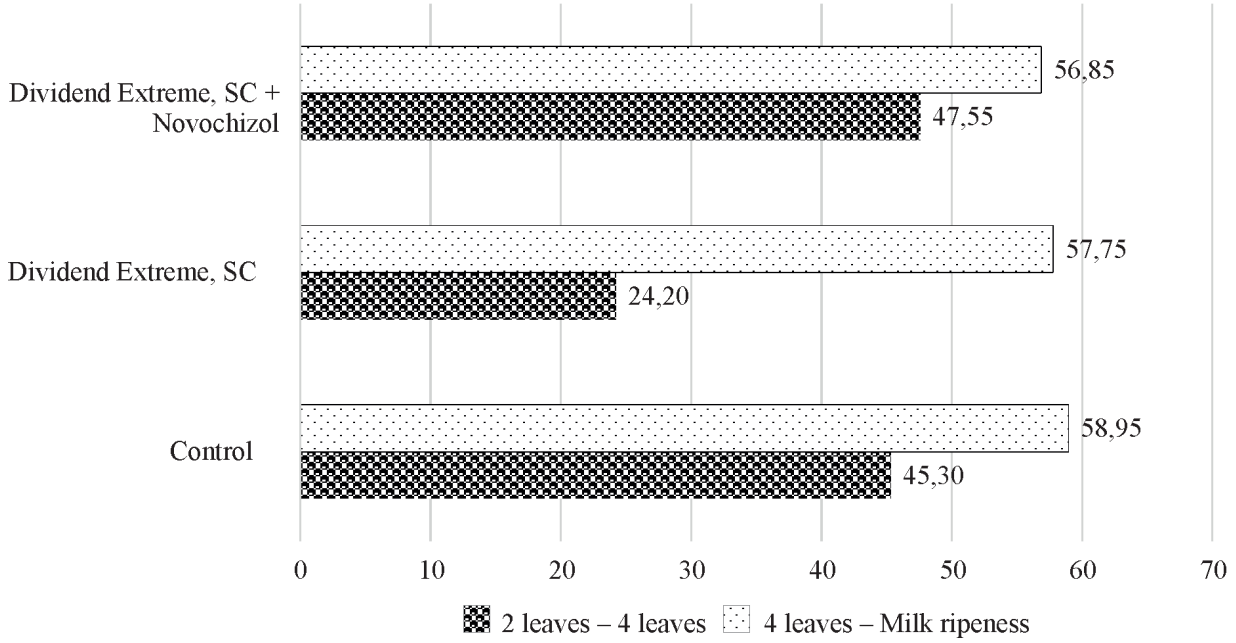
**t_{table} = 2,45 (5% level of significance).

the number of spores decreased by 27.5 and 33.0%, after the use of the experimental composition – by 21.8–25.4% (control – 195.00 ± 5.40 and 1187.50 ± 12.67 conidia / 1 g of soil). The data obtained show that both treatment options can create a phytosanitary effect, but its enhancement at the vegetative stage of plant development is largely facilitated by seed protection with the commercial fungicide Dividend Extreme, SC (see the figure).

A positive aftereffect of protective measures was also noted at the generative stage, when, against a high infection background of *B. sorokiniana*, the frequency of occurrence of affected plants grown from the seeds treated with Dividend Extreme, SC, decreased from 90.97–97.03% (control) to 68.04–81.94% (1.2–1.3 times). Treatment with a composition containing Novochizol reduced the frequency of occurrence of affected plants to a lesser extent – by 1.1 times. Most likely, the decrease in the efficiency of the ecologized mixture was due to a decrease in the amount of difenoconazole and mefenoxam in its composition. More effective suppression of the phytopathogen on seeds was noted when they were treated with the recommended dose of the preparation (0.5–0.6 l/t) (see Table 3).

During the years of the study, the biological efficiency of Dividend Extreme, SC reached 93.3 and 100.0%, respectively, with an infestation in the control of 15.0 and 40.0%. A 13.4-fold decrease in the amount of active ingredient of the fungicide weakened the protection effect by half. The biological efficiency of the composition with Novochizol was inferior to the commercial seed treatment agent with both reduced and increased application rates. The low level of infestation with *Fusarium* spp. fungi (3.0% in 2021, 0.0% in 2022) did not allow us to evaluate the effect of their suppression. A tendency for the effectiveness of seed treatment to go down with a decrease in the application rate of Dividend Extreme, SC was observed against both *Alternaria* spp. (by 1.6 times) and *Penicillium* spp. (by 1.5 times). In 2022, storage molds were not detected in the seed lots. Alternaria infestation in the control was 55.0%. At the same time, the biological effectiveness of Dividend Extreme, SC at the recommended application rate against *Alternaria* spp. reached 100.0%.

In both seasons, seed treatment had virtually no effect on powdery mildew development. Its prevalence was high: 86.56% in the control, 90.49% when treated with Dividend Extreme, SC, 88.54% when treated with the composition with Novochizol. The intensity of leaf colonization differed slightly between the experimental variants, but significantly between the years. An



Накопление конидий *B. sorokiniana* в прикорневом слое (полевой опыт, 2021, 2022 гг.), %
Accumulation of *B. sorokiniana* conidia in the root layer (field experiment, 2021, 2022), %

Табл. 3. Влияние нормы расхода препаратов на эффективность обработки семян (метод рулонов, 26 °С, 2021, 2022 гг.)

Table 3. The influence of the application rate on the effectiveness of seed treatment (roll method, 26 °C, 2021, 2022)

Option	Consumption rate, l/t	*B. sorokiniana*	*Fusarium* spp.	*Alternaria* spp.	*Penicillium* spp.	Total infected, %	Healthy, %
Control	–	15,0	3,0	22,0	6,0	66,0	34,0
Dividend Extreme, SC	0,5	1,0* / 93,3	2,0 / 33,3	9,0 / 59,1	0 / 100,0	12,0	88,0
	0,04	8,0 / 46,7	5,0 / 0	14,0 / 36,4	2,0 / 66,7	29,0	71,0
Dividend Extreme, SC + Novochizol + succinic acid	0,5	2,0 / 86,7	2,0 / 33,3	20,0 / 9,1	4,0 / 33,3	28,0	72,0
	6,7	10,0 / 33,3	0 / 100,0	23,0 / 0	0 / 100,0	32,0	68,0

*The numerator indicates the fungal infestation (%), in the denominator – biological efficiency (%).

increase in the intensity of powdery mildew development was recorded in a dry year: when the seeds were treated with Dividend Extreme, SC – by 2.2 times, with the composition with Novochizol – by 3.5 times, in the control – by 2.3 times (see Table 4).

In the dry 2022, the prevalence and intensity of septoriose and brown rust development were lower compared to the moderately moisture-deficient 2021. In the control, they decreased by 5.4 and 5.8 times for septoriose, 5.9 and 30.3 times for brown rust, in the variant with seed treatment with Dividend Extreme, SC – by 2.1 and 1.9 times (septoriose), 6.3 and 20.4 times (brown rust), when treated with a composition with Novochizol – 2.2 and 3.8 times (septoriose), 2.4 and 6.4 times (brown rust).

Based on the presented data, it can be concluded that the phytosanitary situation in crops formed from the seeds treated with a composition containing Novochizol has worsened. At the same time, the prevalence of septoriose and

Табл. 4. Развитие аэрогенных инфекций в посевах (полевой опыт, молочная спелость, флаг-лист), %
Table 4. Development of aerogenic infections in crops (field experiment, milk ripeness, flag leaf), %

Option	Year	Disease prevalence			Intensity of disease development		
		powdery mildew	septoriose	brown rust	powdery mildew	septoriose	brown rust
Control	2021	77,01	91,98	89,01	4,87	4,81	4,55
	2022	96,02	16,99	15,03	11,31	0,83	0,15
	Average	86,56	54,48	52,02	8,09	2,82	2,35
Dividend Extreme, SC, consumption 0,6 l/t	2021	82,98	88,92	88,92	4,61	3,80	3,67
	2022	98,01	43,05	14,02	10,38	2,05	0,18
	Average	90,49	65,99	51,47	7,50	2,93	1,93
Dividend Extreme, SC + Novochizol + succinic acid, consumption 0,6 l/t	2021	82,00	80,01	87,97	3,90	5,36	3,95
	2022	95,07	36,03	37,04	13,52	1,40	0,62
	Average	88,54	58,02	62,51	8,71	3,38	2,29

brown rust increased by 2.1 and 2.5 times (36.03 and 37.04%, respectively, in the control – 16.99 and 15.03%).

Thus, the decrease in the amount of active ingredients of Dividend Extreme, SC in combination with Novochizol, on the one hand, stimulates the growth of the host plant (in the milk ripeness phase, the height of the plants and the value of their aboveground biomass exceeded the control by 6.21 cm and 29.0%), but on the other hand, it contributes to an increase in the prevalence and intensity of the development of airborne diseases. In both variants with seed treatment, an increase in the density of standing and grain content of the main spike was recorded, but in a dry year, the treatment had a negative effect on the filling of wheat grains, the weight of 1000 grains of which significantly decreased. Seed protection increased the yield in a moderately moisture-deficient season (Dividend Extreme, SC – by 0.16 t / ha, the composition with Novochizol – by 0.34 t / ha) and decreased it in an extremely dry season (by 0.10 and 0.31 t / ha, respectively).

CONCLUSION

The effect of treating spring soft wheat seeds with the fungicide Dividend Extreme, SC and a composition containing Novochizol, Dividend Extreme, SC and succinic acid was studied. Their ability to suppress the development and spread of seed, soil and airborne infections was determined.

Laboratory studies have shown that high biological efficiency (93.3 in 2021, 100.0% in 2022, 15.0 and 40.0% in the control) of wheat seed protection from the main pathogen of common root rot *B. sorokiniana* can be ensured by treatment with Dividend Extreme, SC at the recommended application rate (0.5–0.6 l/t). A reduced application rate weakened the protection effect by half. A tendency for the effectiveness of seed treatment to decrease with a decrease in the application rate of Dividend Extreme, SC was manifested in relation to *Alternaria* spp. (by 1.6 times) and *Penicillium* spp. (by 1.5 times). In terms of its effectiveness, the composition with Novochizol was inferior to the commercial seed treatment agent with both reduced and increased application rates.

The studied preparations reduced the level of soil infectious load of *B. sorokiniana* in the field, but at the vegetative stage of plant development, the reduction of the infectious load was largely facilitated by treatment with a commercial fungicide, which reliably controlled the development and spread of common root rot during the vulnerable growth period (seedlings - two-leaf phase). When treating wheat seeds with a

composition containing Novochizol (consumption rate of 0.6 l / t), the fungicidal effect at the level of a commercial seed treatment agent was manifested only in one of the seasons. The effect of seed treatment persisted at later stages of development. In the four-leaf phase, the biological efficiency of Dividend Extreme, SC was 29.8 and 55.0% in 2021 and 2022. The composition with a reduced content of a.i. Dividend Extreme, SC + Novochizol showed the same result in both years (biological efficiency was 31.0 and 34.0%, respectively). At the stage of milk ripeness, a decrease in the development of common root rot was observed in both variants with treatment with greater efficiency from dressing with a commercial fungicide.

During observations of the development of airborne diseases, it was noted that seed treatment with a composition containing Novochizol under conditions of insufficient atmospheric moisture indirectly (through growth stimulation of the host plant) affected the increase in leaf damage by both septoriose and brown rust (by 2.1 and 2.5 times).

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Идентификация патогенов томата *de novo*

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Одной из наиболее подверженных заболеваниям культур является томат (*Solanum lycopersicum*), что создает необходимость максимально точного и полного определения вызывающих эти болезни патогенов. Метод, позволяющий определить патогены с нуля или их комплекс, – секвенирование высококонсервативного локуса ДНК. Цель работы – определение видового состава патогенных грибов и бактерий данным методом. Исследование проводилось в 2023 г. в Бахчисарайском районе. Отбор материала проводился с растений томата сорта Pink Heart (Китай), выращенных в условиях защищенного грунта. При визуальном осмотре на растениях были обнаружены два типа поражений, из которых в чистую культуру было выделено 15 изолятов бактерий и грибов на среды LB, горохово-морковный агар и картофельно-декстрозный агар. Из выросших культур отбирались образцы, обладающие схожими признаками для выделения тотальной ДНК, на которой ставилась ПЦР с универсальными грибными и бактериальными праймерами. Полученные ампликоны секвенировались. С первого типа поражения были получены нуклеотидные последовательности, гомологичные *Enterobacter ludwigii, E. kobei, E. cloaca* и *Pseudomonas veronii* у бактерий. Идентичность полученных гомологов составляла 100% при 100%-м покрытии. У грибов с этого же типа поражений нуклеотидные последовательности гомологичны *Botrytis cinerea* и роду *Penicillium*, их гомологичность составила, как и у бактерий, 100% со 100%-м покрытием. Со второго типа поражения получены последовательности, гомологичные *Cladosporium* sp. и *Penicillium chrysogenum.* Идентичность гомологов составила 100% со 100%-м покрытием.

Ключевые слова: томат сорта Pink Heart, грибные и бактериальные патогены, праймеры на 16 S, ITS праймеры, секвенирование

Identification of tomato pathogens *de novo*

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One of the most disease-prone crops is tomato *(Solanum lycopersicum),* which creates the need to identify the pathogens causing these diseases as accurately and completely as possible. One of the methods allowing to determine pathogens from scratch or their complex is sequencing of highly conserved DNA locus. The purpose of the work was to determine the species composition of pathogenic fungi and bacteria by this method. The study was conducted in 2023, in the Bakhchisaray district. The material was selected from tomato plants of Pink Heart variety (China) grown under protected soil conditions. During visual inspection, two types of lesions were detected on the plants, from which 15 isolates of bacteria and fungi were isolated into pure culture on LB medium, pea-carrot agar and potato-dextrose agar. From the grown cultures, samples with similar features were selected for isolation of total DNA, on which PCR with universal fungal and bacterial primers was performed. The amplicons obtained were sequenced. Nucleotide sequences homologous to *Enterobacter ludwigii, E. kobei, E. cloaca* and *Pseudomonas veronii* in bacteria were obtained from the first lesion type. The identity of the obtained homologues was 100% at 100% coverage. In fungi from the same type of lesions, the nucleotide sequences were homologous to *Botrytis cinerea* and the genus *Penicillium,* their homologues

were, as in bacteria, 100% with 100% coverage. Sequences homologous to *Cladosporium* sp. and *Penicillium chrysogenum* were obtained from the second lesion type. The identity of the homologues was 100% with 100% coverage.

Keywords: tomato variety Pink Heart, fungal and bacterial pathogens, 16 S primers, ITS primers, sequencing

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The authors declare no conflict of interest.

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INTRODUCTION

In connection with the food security policy, vegetable growing has been developing in Russia in the 2010s–2020s. From 2013 to 2019, the production volume of greenhouse vegetables alone increased from 1.15 to 2.01 million tons [1, 2]. Among other crops, this process also affected tomato production. Thus, in 2021–2023 alone, the gross tomato harvest in the Russian Federation increased by 5.7% (from 1.07 to 1.13 million tons) [3]. However, tomatoes, like other crops, are susceptible to more than 200 different bacterial and fungal diseases. The resulting crop losses can reach 30–100% [4–6]. In this regard, the problem of determining the species of plant pathogens remains relevant, especially in cases where it is difficult to phenotypically determine their species and select plant protection methods. In modern developments, various methods are used for this purpose, but in practice, the most widely used method is PCR for a specific gene or multiplex PCR. Both methods are intended primarily to establish the presence of a certain type of pathogen on a plant [7–12]. However, in some cases, it is necessary to determine the type of pathogen or their combination on a plant from scratch (de novo). Sequencing of a highly conserved locus is often used for this purpose. This method allows for taxonomic identification of most groups of organisms to the genus and species level [11–14]. However, in the absence of a sufficient number of reference sequences in databases, determination to the species level may be difficult [14].

The purpose of the study is to identify the species composition of pathogenic fungi and bacteria that affect tomato plants using sequencing and universal primers.

The objectives of the study included an isolation of phytopathogens from the affected parts of the tomato into a pure culture; an isolation of the DNA of phytopathogens and conducting a sequencing reaction of a highly conserved region; and a comparison of the obtained sequences with the reference sequences from the GenBank database.

MATERIAL AND METHODS

The plant material for the study was collected in the village of Ayvovoye (Republic of Crimea) from tomato plants of the Pink Heart variety (China) grown in protected soil conditions (see Fig. 1). The affected plants were from the summer-autumn rotation. They were sown

in July in the cassettes with stone wool, and in the seedling phase they were transplanted into the brackets with stone wool. Monthly seedlings together with the brackets were transferred to the mats with coconut substrate, 4 pcs. per mat. During the entire vegetation period, complete mineral nutrition was maintained (solution pH 5.5) in accordance with the phase of plant development with drainage of all unused nutrient solution. Selection of the affected material was carried out in the fruit ripening stage of the second-third raceme (November). Phytopathogens from the affected leaves, stems and fruits were isolated in pure culture according to the method[1]. DNA was isolated by the CTAB method[2].

Рис. 1. Место отбора материала – теплица защищенного грунта, томат сорта Pink Heart

Fig. 1. Place of sampling – greenhouse with protected soil, Pink Heart tomato variety

Universal primers ITS1/ITS4[3] (for fungi) and of the 16S rRNA gene: fD1 and rD1[4] (for bacteria) were used for PCR and sequencing.

The reaction was carried out in a Gene Explorer thermocycler (BIOER, China) according to the following program: for bacteria – initial denaturation (95 °C, 5 min), 30 cycles, including denaturation (94 °C, 30 sec), annealing (55 °C, 30 sec) and elongation (72 °C, 1 min); for fungi – initial denaturation (94 °C, 2 min), 35 cycles, including denaturation (94 °C, 30 sec), annealing (55 °C, 30 sec) and elongation (72 °C, 1.5 min). Sequencing was carried out at Eurogen JSC on a 3500xL Applied Biosystems genetic analyzer (Thermo Scientific, USA). The nucleotide sequences obtained as a result of sequencing were compared with the sequences deposited in GenBank using the BLAST service.

RESULTS AND DISCUSSION

Two types of lesions were observed on the plants. The first type of lesions on the plants were characterized by spotting on the leaves, stems and fruits. On the leaves, the spots were large, of an indefinite shape, and dark brown in color. The lesions formed on the tip of the leaf blade and gradually captured the entire surface of the leaf, leading to its wilting.

The stem was surrounded by dark spots of varying heights. When a closed ring-shaped lesion formed on the stem, its top began to wither.

The fruits were covered with dark brown spots, the damage spreading from the top of the fruit. The sepals were also affected, with the tips darkening first (see Fig. 2).

The second type of damage to plants was characterized only by spotting on the leaves. Very small, pale, light yellow spots of a round

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Puc. 2. Первый тип поражения
Fig. 2. Type one lesion

shape with a blurred border were scattered over the entire surface of the leaf blade. Over time, they darkened, acquiring a more brown color, and their borders became clear (see Fig. 3).

The lesions found on the plants did not allow us to accurately determine the disease and the species of pathogenic organisms, so we decided to isolate the phytopathogens into a pure culture in order to identify them using molecular methods. A total of 15 isolates of fungi and bacteria were isolated from the plants into a pure culture, of which eight isolates were isolated from the first type of lesions on LB and pea-carrot agar media, and seven isolates from the second type of lesions on potato-dextrose agar.

Strains that were present in the majority of replicates and had similar morphological features to other isolates when isolated in pure culture were selected for sequencing. A total of three bacterial isolates and six fungal isolates were sequenced.

According to the sequencing results, the bacteria isolated from the first type of lesion are homologous to *Enterobacter ludwigii*, *E. kobei*, *E. cloaca* and *Pseudomonas veronii*, the identity of all homologues is 100% at 100% coverage (see Table 1). According to the literature, strains of *E. ludwigii*, *E. kobei*, *E. cloaca* are defined as plant pathogens[5].

At the same time, according to literature data, *P. veronii* is not a pathogen and can be used in biotechnology as a destructor of some hydrocarbons [15]. It is noted that the nucleotide sequence of isolate LBst.3.2.2 has a high percentage of similarity with the nucleotide sequence of isolate LBst.1.2.2, homologous to several strains of *Enterobacter*, which suggests that the lesion is caused by *Enterobacter ludwigii.*

[5]*Humann J.L., Wildung M., Cheng C.H., Lee T., Stewart J.E., Drew J.C., Triplett E.W., Main D., Schroeder B.K.* Complete genome of the onion pathogen Enterobacter cloacae EcWSU1 // Stand Genomic Sci., 2011, vol. 5, N 3, pp. 279–286. DOI: 10.4056/sigs.2174950.

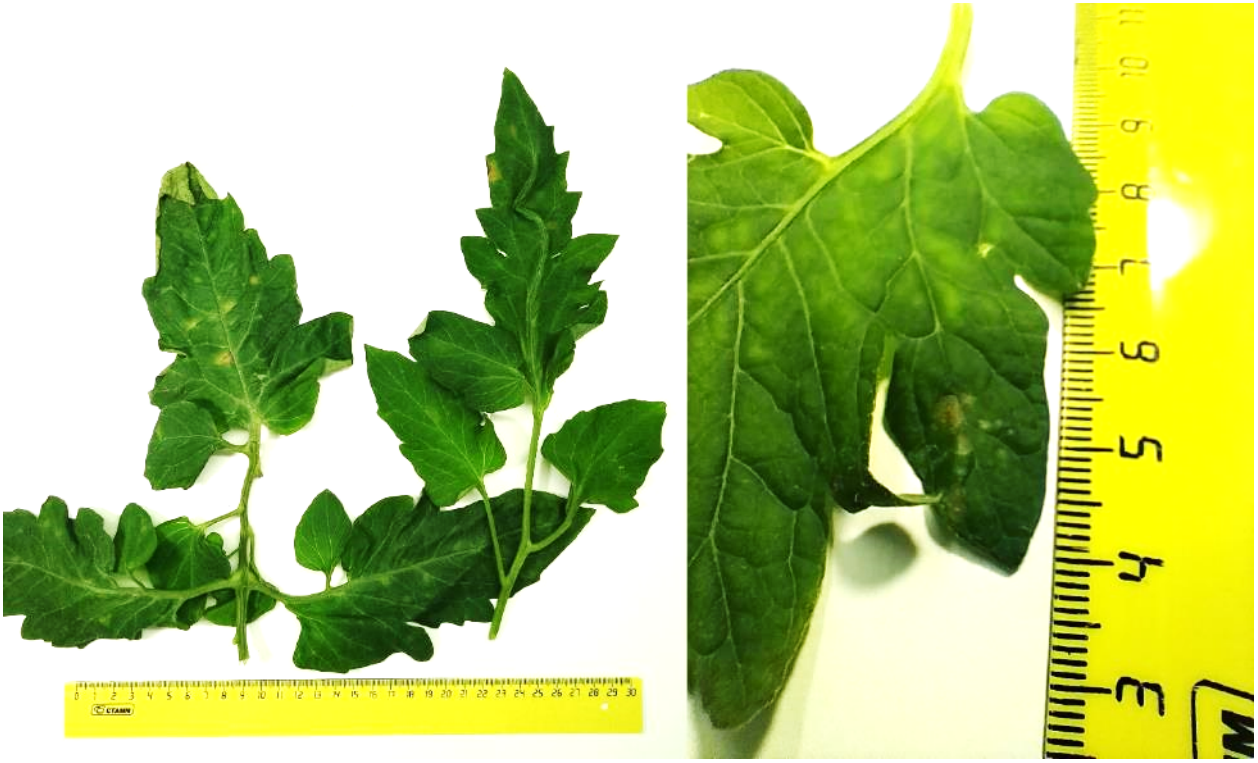

Рис. 3. Второй тип поражения
Fig. 3. Type two lesion

Fungi with nucleotide sequences homologous to Botrytis cinerea and species of the genus *Penicillium* were also found on the first type of damage. According to the literature, *B. cinerea* is a common pathogen of tomato and other cultivated plants, while representatives of the genus *Penicillium* are mostly not pathogens of living plants. Since *B. cinerea* is a necrotrophic phytopathogen and prefers to infect plants with already damaged or soft tissues [16, 17], it can be assumed that the primary damage could have been caused by *Enterobacter* bacteria. Also, damage by bacteria could have created conditions for the development and spread of fungi of the genus *Penicillium*. The homology of fungal isolates was, as with bacteria, 100% with 100% coverage (see Table 1).

From the second type of lesion, only fungi homologous to *Cladosporium* sp. and *Penicillium chrysogenum* were isolated. The homologues were 100% identical with 100% coverage (see Table 2). Since, according to literature data, pathogenic properties are known only for *Cladosporium* of these fungi, it is presumably the cause of the lesion.

Табл. 1. Оценка гомологии ДНК изолятов, выделенных с поражений первого типа, последовательностям из базы данных GenBank

Table 1. Assessment of the DNA homology of the isolates obtained from type one lesions with sequences from the GenBank database

Isolate	Sequence length, b.p.	Homologs
LBст.1.2.2	663 (FD) 594 (RD)	*Enterobacter ludwigii*
		Enterobacter kobei
		Enterobacter cloacae
LBст.3.2.2	660	*Enterobacter ludwigii*
LBп.1.2.1	1412	*Pseudomonas veronii*
HML-B_л.1.2white	527	*Penicillium* sp.
HMл.3.1gray.2	448	*Botrytis cinerea*
HMст.3.1gray-green	514	*Didymella pinodella*
		Penicillium sp.
		Alternaria alternata

Note. Homology, coverage/identity – 100/100%.

Табл. 2. Оценка гомологии ДНК изолятов, выделенных с поражений второго типа, последовательностям из базы данных GenBank

Table 2. Assessment of the DNA homology of the isolates obtained from type two lesions with sequences from the GenBank database

Isolate	Sequence length, b.p.	Homologs
КДл.4black2	491	*Cladosporium* sp.
КДл.4white1	520	*Penicillium chrysogenum*
КДл.1 black1	485	*Cladosporium* sp.

Note. Homology, coverage/identity – 100/100%

CONCLUSION

Sequencing of highly conserved genome regions such as 16S (for bacteria) and ITS (for fungi) allows us to determine *de novo* the set of species living on the plant and to isolate pathogenic organisms among them. Comparing the obtained nucleotide sequences with sequences from the GenBank database, we were able to assume that the first type of damage is caused by *E. ludwigii* bacteria, which affected the stem and its vascular bundles. The second type of damage on the leaves is caused by fungi of the genus *Cladosporium* sp.

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Влияние комплексного паразитоцида на динамику зараженности овец гельминтами

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Целью исследования являлась оценка влияния комплексного терапевтического противопаразитарного средства группового применения ПКГ-АА на сезонное течение основных инвазий у овец Горного Алтая. Копрологическими исследованиями установлено, что зараженность овец гельминтами подотряда Strongylata в течение года составила 65,0–100,0%, мониезиями – 55,0, легочными стронгилятами – 36,0, трихоцефалами и дикроцелиями – по 35,0%. Среднегодовой уровень экстенсивности инвазии желудочно-кишечными стронгилятами достигал 77,4%, трихоцефалами – 26,2, протостронгилами – 25,4, мониезиями – 12,4, дикроцелиями – 8,4%. На долю нематод в структуре гельминтокомплекса приходилось 84,2%, трематод – 8,8, цестод – 7,0%. Полностью доминировали желудочно-кишечные стронгиляты (47,9%), в значительном количестве были представлены трихоцефалы (21,1%) и легочные стронгиляты (15,2%). Установлено, что противопаразитарные зернофуражные гранулы ПКГ-АА эффективны против всех основных групп гельминтов овец. При трихоцефалезе и дикроцелиозе эффективность составила 100,0%, при желудочно-кишечных стронгилятозах – 98,3, легочных стронгилятозах – 93,0, мониезиозе – 91,0%. Показано, что в овцеводческих хозяйствах региона при благоприятной эпизоотической обстановке по основным гельминтозам может быть реализована схема двукратного (весна и осень) применения комплексных терапевтических средств, обладающих широким спектром паразитоцидного действия. Применение препаратов позволило удерживать численность паразитов и зараженность ими животных на относительно низком уровне. Животные уходили на зимне-стойловое содержание с достаточно низкими показателями экстенсивности (ЭИ = 10,0–20,0%) и интенсивности заражения (ИО = 14,7–18,9 яиц/личинок в 1 г фекалий).

Ключевые слова: гельминты, овцы, зараженность, структура гельминтокомплекса, паразитоциды, эффективность

Influence of complex parasitocide on the dynamics of helminth infestation of sheep

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The purpose of the study was to evaluate the effect of complex therapeutic antiparasitic means of group application of AFG AA on the seasonal development of the main infestations in sheep of the Altai Mountains. Coprological studies have established that the infestation of sheep with helminths of the suborder Strongylata during the year amounted to 65.0–100.0%, moniezia – 55.0, pulmonary strongylates – 36.0, whipworms and dicrocelia – 35.0% each. The average annual intensity of invasion by gastrointestinal strongylates reached 77.4%, whipworms 26.2, protostrongylus 25.4, moniezia

12.4, and dicrocelia 8.4%. Nematodes accounted for 84.2% of the helminth complex, trematodes – 8.8, cestodes – 7.0 %. Gastrointestinal strongylates were completely dominant (47.9%), whipworms (21.1%) and pulmonary strongylates (15.2%) were represented in significant numbers. It was found that antiparasitic grain-forage granules AFG AA are effective against all major groups of sheep helminths. In whip-worm infection and dicroceliosis, the efficiency was 100.0%, gastrointestinal strongylatosis – 98.3, pulmonary strongylatosis – 93.0, monieziasis – 91.0%. It was shown that in sheep farms in the region, under favorable epizootic conditions for main helminthiases, a scheme of double (spring and autumn) use of complex therapeutic agents with a wide parasitocidal spectrum of action can be implemented. The use of drugs made it possible to keep the number of parasites and the infection of animals by them at a relatively low level. Animals we kept during winter stall-feeding period with fairly low indicators of extensiveness (IE = 10,0–20,0%) and intensity (nidus index = 14.7–18.9 eggs/larvae per gram of feces) of infection.

Keywords: helminths, sheep, infection, structure of the helminth complex, parasitocides, effectiveness

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INTRODUCTION

Farms of all types of ownership located in the Altai Republic contain about 500 thousand sheep and goats. Due to the high number of animals on pastures, the epizootic situation is naturally becoming more complicated, including invasive diseases. When determining the approach to organizing treatment and preventive measures, it should be taken into account that various types of helminths can dominate in the parasitocoenosis of sheep. In this regard, there is a need for constant monitoring of the epizootic situation.

Despite the availability of knowledge in the field of epizootology of helminthiasis in sheep, the effectiveness of new antiparasitic agents and methods of their application, there are currently few studies concerning population patterns of helminth development, in particular changes in their numbers under the influence of regular antiparasitic treatments.

Quite a lot of information has been accumulated about the composition of the helminth fauna, the degree of infection of sheep with helminths, and the characteristics of the invasive process in flat and mountainous areas of various regions of the planet[1] [1–4]. Research on Siberia, including the Altai Republic, is fragmentary, and individual issues require more detailed study (primarily, the impact of anthropogenic pressure on the course of parasitic invasions). The causative agents of parasitic invasions of sheep in the Altai Mountains are characterized by a wide species diversity and are represented by the main taxa of parasitic invertebrates. The most significant in epizootic terms are gastrointestinal and pulmonary strongylates, moniezia, dicrocoelia, sheep tick and sheep gadfly. In most cases, representatives of these taxa parasitize simultaneously in the form of mixed invasions, so the use of drugs with a wide range of antiparasitic activity is jus-

[1]*Tsepilova I.I., Shemyakova S.A., Nikolaeva E.A.* Helminth fauna of sheep in the Teberda River valley // Theory and practice of combating parasitic diseases: collection of scientific articles based on the materials of the international scientific conf. Moscow, 2022, vol. 23, pp. 484–489.

tified. The use of complex agents allows you to act on the entire spectrum of parasites, which helps reduce the volume of their use and the frequency of manipulations with animals[2, 3] [5, 6].

The purpose of the study is to assess the effect of a complex antiparasitic agent on the seasonal course of the main invasions in sheep of the Altai Mountains.

The study included the determination of the structure of the helminth complex, the seasonal dynamics of animal infection rates and the therapeutic efficacy of the antiparasitic agent, which was used as grain fodder antiparasitic feed granules (AFG).

MATERIAL AND METHODS

The study was conducted in 2023 and 2024 on a flock of sheep of the Gorno-Altai Selection and Breeding Center in the Ongudai District of the Altai Republic. The farm collected coprological samples for ovolarvoscopic studies with subsequent characterization of the level and structure of animal infestation. Surveys were carried out at least once every 2 months (20–25 samples). When studying the infestation of sheep with helminths of the gastrointestinal tract, the helminthoscopy method according to Kotelnikov-Khrenov was used, for pulmonary helminths –the helmintholarvoscopy method according to Berman-Orlov, for dicrocoelia – the method of successive washes[4].

To determine the effect of a complex parasitocidal agent on the seasonal course of the main invasions in sheep, we used grain forage granules with avermectin C and albendazole (AFG AA) prepared on a AShG 250 extruder in the experimental shop of the Gorno-Altai Research Institute of Agriculture. Albendazole and avermectin C have pronounced activity against nematodes, flatworms and parasitic insects of the main taxa of the parasite complex of sheep in the region. The granules were fed from feeders once to 380 sheep in spring (May) and autumn (October) in groups at the rate of 2.5 g / kg of animal weight (avermectin C – 0.2 mg / kg, albendazole – 5.0 mg / kg).

Before assessing the effectiveness of the preparation, helmintho-ovoscopic examinations were carried out. The control in the experiment was the infection rate of animals before treatment. The parasitocidal effectiveness was assessed using the "critical test" method by examining coprological samples using ovolarvoscopy methods 20 days after the application of the preparation. Based on the results of coprological examinations, the following infection indices were determined: EI, % – extensiveness of invasion or prevalence, proportion of infected animals; AI – abundance index, arithmetic mean of the number of eggs/larvae in 1 g of feces per examined animal. Based on the EI values of individual taxa, the indices of the structural index of the helminth complex[5], levels of extensiveness and intensity of infection (average values of EI and AI indices in samples) were calculated. The following indices were used to assess the parasiticidal activity of the preparation:

EE, % – extensive efficiency, the proportion of animals completely freed from parasites, in relation to the control (untreated); IE, % – intensive efficiency, a decrease in the average number of eggs in relation to the control after deworming. The number of moniesia eggs in the samples was conditionally accepted as an indirect, variable indicator of infestation.

To compare the parasiticidal efficiency of AFG, statistical analysis of data was performed on the geometric mean value of the number of helminth eggs. Comparison of the differences

[2]*Engasheva E.S., Moskalov V.G., Muromtsev A.B.* Efficiency of the drug Monizen forte in helminthiasis and arachnoentomoses of sheep // Theory and practice of combating parasitic diseases: collection of scientific articles based on the materials of the international scientific conf. Moscow, 2019, vol. 20, pp. 205–209.

[3]*Lagereva E.V., Abramov V.E., Musaev M.B.* Supramolecular anthelmintic complex Altrik-extra for the treatment and prevention of the main helminthiases of animals // Theory and practice of combating parasitic diseases: collection of scientific articles based on the materials of the international. scientific conf. Moscow, 2020, vol. 21, pp. 187–193.

[4]*Kotelnikov G.A.* Helminthological studies of animals and the environment: a handbook. Moscow: Kolos, 1984. 208 p.

[5]*Marchenko V.A., Efremova E.A., Vasilyeva E.A.* Structure of helminthocenosis of cattle in the Altai Mountains // Russian Journal of Parasitology, 2008, N 3, pp. 18–23.

between the control and experimental groups was carried out on the basis of the parametric t-criterion at a significance level of $p \leq 0,05$ [7].

RESULTS AND DISCUSSION

Dynamics and structure of sheep infection with helminths

Table 1 shows the seasonal dynamics of helminth infestation in sheep based on the results of surveys conducted 50 days after parasiticidal treatments in May and October. Infestation with gastrointestinal helminths of the Strongylata suborder during the specified period was within 20.0–100.0% with AI = 9.5–637.9. For helminths of the genus *Nematodirus*, the considered indicators were 10.0–60.0% and 2.3–43.7 eggs, respectively, for whipworms and intestinal nematodes of the genus *Trichocephalus* – 20.0–35.0% and 2.5–11.4 eggs. Moniezia and Dicrocelium eggs began to be registered in the summer-autumn period. Moniezia infestation reached 55.0% with AI = 64.6. The maximum EI of Dicrocelium was 35.0%, the average number of eggs was 20.5 specimens. Infestation of sheep with pulmonary strongylates, which were represented by the genus *Protostrogylus*, was at a relatively low level throughout the year (6.7–36.0% with AI = 2.5–9.3). The average annual EI of intestinal strongylates was 65.3%, whipworms – 24.5, protostrongyles – 22.2, moniezia – 12.4, dicrocoelia – 6.5%.

Structure of the helminth complex

Knowledge of the structure of the helminth complex allows for rational construction and optimization of the system of antiparasitic measures. According to the results of ovolarvoscopic studies, the share of nematodes in the annual structure of the helminth complex is 84.2%,

Табл. 1. Динамика зараженности овец гельминтами (2023, 2024 гг.)
Table 1. Dynamics of helminth infection of sheep (2023, 2024)

Date of examination	*n*	Strongylata*		*Nematodirus*		*Trichocephalus*		*Moniezia*		*Dicrocelium*		*Protostrongylus*	
		EI, %	AI	EI, %	AI	EI, %	AI	EI, %	AI	EI, %	AI	EI, %	AI
January	20	73,3	183,0	60,0	43,7	26,6	11,4	0	0	0	0	20,0	9,0
March	20	70,0	101,6	25,0	6,2	35,0	6,6	0	0	0	0	25,0	6,0
April	20	65,0	105,2	20,0	5,1	35,0	10,7	0	0	0	0	20,0	5,0
May	20	78,3	187,6	20,0	3,8	35,0	7,6	0	0	0	0	20,0	4,0
July	20	75,0	330,3	10,0	2,3	30,0	8,1	25,0	22,7	0	0	25,0	8,5
August	25	80,0	445,5	24,0	18,5	32,0	6,8	32,0	56,5	24,0	20,5	32,0	9,3
October	20	100,0	637,9	30,0	21,6	20,0	4,6	55,0	64,6	35,0	19,5	36,0	7,0
December	15	20,0	9,5	13,3	6,9	0	0	0	0	0	0	6,7	2,5
January	20	26,6	29,2	20,0	7,1	6,6	2,5	0	0	0	0	15,0	6,5
Average	–	65,3	225,5	24,7	12,5	24,5	6,5	12,4	15,9	6,5	4,4	22,2	6,4

* Intestinal strongylates without nematodes.

trematodes - 8.8, cestodes - 7.0% (see Fig. 1). Representatives of gastrointestinal strongylates absolutely dominated in terms of total values of structural indices (47.9%), whipworms (21.1%) and pulmonary strongylates (15.2%) were significantly represented. The study data indicate that during antiparasitic measures, the main attention should be paid to monieziasis, intestinal and pulmonary strongylatoses, which involves the use of drugs with nematode- and cestodocidal action.

Parasitocidal efficacy of the drug

The least expensive of the complex preparations are AFG AA granules, which are mainly focused on intestinal and pulmonary strongylata, monieziasis and arachnoentomoses. Group feeding is a low-cost and effective technology for controlling the number of parasitic species. It has been established that AFG AA is quite effective against the main groups of sheep helminths (see Table 2). Thus, the extensive effectiveness against gastrointestinal strongylata was 93.7 (May) and 92.0% (October), the intensive effectiveness was 98.3 and 97.9%, respectively. The preparation showed 100% effectiveness against whipworms and dicrocoelium. In case of pulmonary invasions, 89.0% of animals were freed from helminths, the number of larvae in feces decreased by 93.0%. In case of parasitism of moniezias, the drug significantly reduced the infection rates: the EE value was 91.0%. Studies by other authors have established that oral administration of drugs based on macrolides and benzimidazoles invariably demonstrates high parasiticidal activity against helminthiasis of farm animals [8–11].

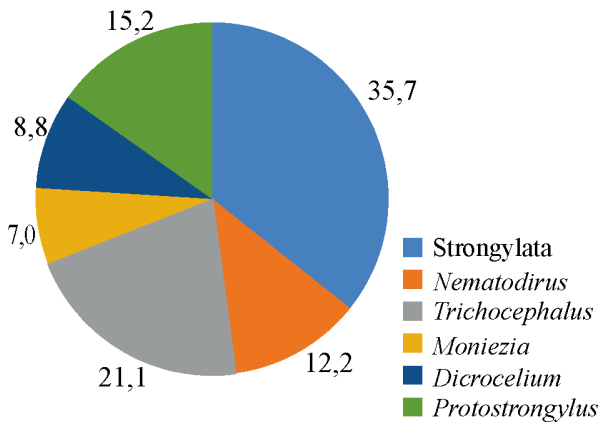

Рис. 1. Структура гельминтокомплекса, %
Fig. 1. Structure of the helminth complex, %

The effect of antiparasitic drug on the number of individual groups of helminths

The level of animal infestation with helminths largely determines the nature of the negative impact on the host organism and, consequently, the need for any antiparasitic measures. The diversity of parasites, which differ in biology and epizootology of the diseases they cause, as well as sensitivity to antiparasitic agents, a priori suggests resorting to appropriate therapeutic drugs and schemes (programs) for their use. The use of complex therapeutic agents with a broad spectrum of action, against the background of a unified approach to their use, can significantly optimize the system of antiparasitic measures.

The implementation of such an approach helps to cut the costs of antiparasitic measures, reduce the volumes of drugs used and the frequency of manipulations with animals. At the same time, regular tracking (monitoring) of the number of parasites is necessary, the results of which regulate the nature of intervention in the parasitic system (choice of drugs, timing and frequency of administration, etc.).

In sheep farms of the region, in a favorable epizootic situation for the main helminthiases, a scheme of double (spring and autumn) application of complex therapeutic agents with a wide spectrum of parasiticidal action (nematodes, cestodes, trematodes, parasitic arthropods) can be implemented.

A scheme of double feeding of antiparasitic grain feed granules AFG AA was tested on a flock of sheep. In the winter-spring period, the EI of various helminths was approximately at the same level, amounting to 80.0% in May (Fig. 2, *a*). In July, 50 days after the drug application, the EI recovered to 75.0% due to the infection of animals on the pasture. At the end of the pasture season (October), all animals (100.0%) were infected with helminths. 64 days after the second antiparasitic therapy, which was carried out in October, the extensiveness of infection of sheep with all helminths was 20.0%, including gas-

Табл. 2. Эффективность противопаразитарных зернофуражных гранул ПКГ-АА при гельминтозах*
Table 2. Efficiency of antiparasitic grain-forage granules AFG-AA against helminthic diseases*

Period of drug feeding	Number of animals, heads		EI, %	Number of eggs/larvae in 1 g of feces		EE, %	IE, %	*Ef*, %	*p*-criterion
	treated	examined		arithmetic mean	geometric mean				
Gastrointestinal strongylates									
May	280	20**/20	78,3/5,0	187,6 ± 21,5/3,2	2,3 ± 0,06/0,50	93,7	98,3	78,3	NA
October	380	20/25	100,0/8,0	637,9 ± 93,1/13,7 ± 8,2	2,8 ± 0,12/1,13 ± 0,07	92,0	97,9	59,7	< 0,05
Whipworms									
May	280	20/20	35,0/0	7,6 ± 3,4/0	0,88 ± 0,12/0	100,0	100,0	100,0	NA
Octorber	380	20/25	20,0/0	4,6 ± 2,5/0	0,66 ± 0,08/0	100,0	100,0	100,0	NA
Moniezia									
Octorber	380	20/25	55,0/5,0	64,6 ± 12,5/4,7	1,8 ± 0,09/0,67 ± 0,07	91,0	92,8	62,8	< 0,05
Dicrocoelium									
Octorber	380	20/25	35,0/0	19,5 ± 4,6/0	1,3 ± 0,15/0	100,0	100,0	100,0	NA
Pulmonary strongylates									
Octorber	380	20/25	36,0/4,0	7,0 ± 3,1/0,5	0,49 ± 0,08/–	88,9	92,9	–	NA

* Ef – geometric mean efficiency; p-criterion – statistical significance of the probability of differences in geometric mean values to control; NA – statistical analysis was not applied.

** The numerator shows the data before treatment with the drug, the denominator shows the data after.

trointestinal strongylates – 20.0%, protostrongylates – 6.7%. Consequently, the animals went into winter with a fairly low value of the extensiveness of infection, although in January there was a slight increase in the indicator (29.4%), probably due to the surviving imaginal and larval forms of parasites.

Fig. 2, *б* shows the dynamics of the intensity of infection of sheep with helminths against the background of the use of AFG AA. In the winter-spring period (January - May), the overall invasion intensity according to the propagative forms of helminths (eggs + larvae) was within 120.4-203.0 specimens; in July, 50 days after the drug application, the AI was 349.2 due to the infection of sheep in the pasture. At the end of the grazing season (October), all animals were infected with helminths. During this period, the AI reached its maximum value of 755.2. In December, 64 days after the second antiparasitic therapy, the abundance index, reflecting the intensity of animal infection, was 18.9 for all groups of helminths, including 18.9 for gastrointestinal strongylates and 2.5 for protostrongylates. Thus, the animals went into winter with a fairly low infection intensity. In January of the following year, a slight increase in the index was recorded (AI = 45,3).

It has been established that double application of complex parasiticides in spring (before going out to pasture) and autumn (before placing livestock in stall barn housing) allows maintain-

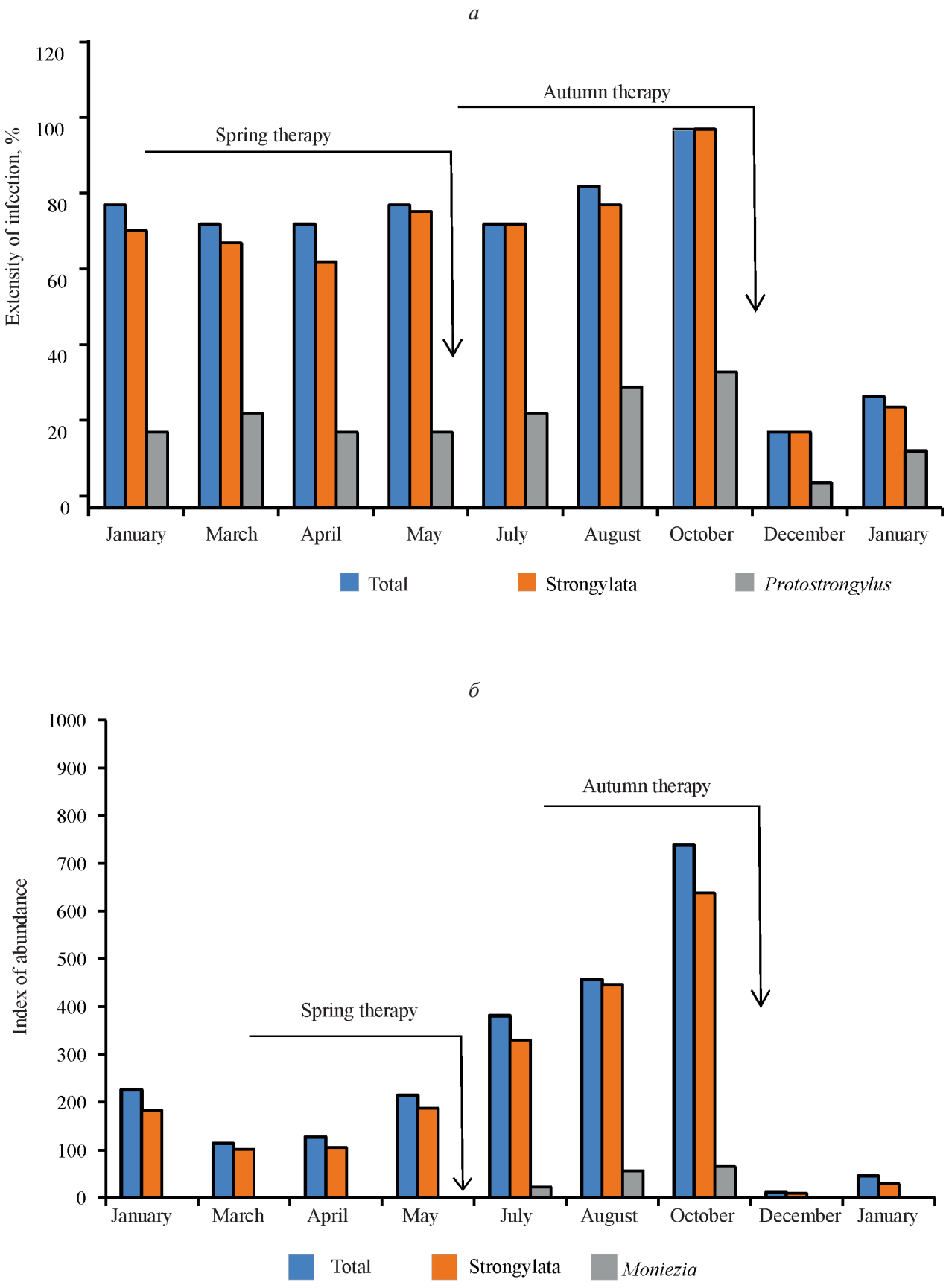

Рис. 2. Динамика экстенсивности (*а*) и интенсивности заражения (*б*) овец гельминтами (2023, 2024 гг.)
Fig. 2. Dynamics of the extensity (*a*) and intensity of infection (*б*) of sheep with helminths (2023, 2024)

ing a relatively low number of helminths (nematodes, cestodes, trematodes), preventing clinical manifestations of helminthiasis and preventing losses in animal productivity. According to clinical indications, additional antiparasitic therapy can be carried out in the first ten days of August for young animals of the current and previous years of birth.

CONCLUSION

In the Ongudai district of the Altai Republic, the infection of sheep with helminths was characterized by the following indicators: 1) suborder Strongylata: EI = 65.0–100.0%, AI = 101.6–637.9; 2) whipworms: EI = 20.0–35.0%, AI = 2.5–11.4; 3) moniezia: EI = 55.0%, AI = 64.6; 4) dicrocoelium: EI = 35.0%, AI = 19.5; 5) protostrongylids: EI = 20.0–36.0%, AI = 4.0–9.3. The average annual level of extensiveness of invasion by intestinal strongylates was 77.4%, whipworms – 26.2, protostrongyles – 25.4, moniezia – 12.4, dicrocoelium – 8.4%. The share of nematodes in the structure of the helminth complex was 84.2%, trematodes – 8.8, cestodes – 7.0%. Gastrointestinal strongylates were absolutely dominant (47.9%), whipworms (21.1%) and pulmonary strongylates were quite common (15,2%).

Antiparasitic grain feed granules AFG AA are very effective against all main groups of sheep helminths. In the event of gastrointestinal strongylosis in case of double (spring and autumn) therapy, the effectiveness of the drug was 97.9 and 98.3% respectively, in case of whip-worm infection and dicrocoeliosis – 100.0%, in case of pulmonary strongylosis – 89.0 and 93.0%, in case of monieziasis – 91.0%. The use of the complex drug allowed to keep the number of parasites and their infestation among animals at a relatively low level. The sheep were kept in winter stalls with fairly low rates of extensiveness (10.0–20.0%) and infection intensity (14.7–18.9 eggs/individual), which allows us to recommend double therapy of animals with AFG AA at a rate of 2.5 g/kg to control infection with the main types of zooparasites in the conditions of the Altai Mountains.

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Межпородные различия гематологических показателей коров в Западной Сибири

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Представлены результаты анализа содержания и изменчивости показателей гематологического статуса у коров разных пород, разводимых на территории Западной Сибири. Продемонстрированы данные по оценке различий абсолютного количества лейкоцитов, эритроцитов, тромбоцитов, концентрации гемоглобина, относительного числа клеток лейкоцитарной формулы, индексов стресса и адаптации между животными голштинской, черно-пестрой, красной степной пород. Исследована периферическая кровь здоровых коров 2-й лактации. Измерение гематологических параметров проводили с помощью автоматического гематологического анализатора PCT 90Vet. Лейкоцитарную формулу подсчитывали в мазках крови, фиксированных в 95-градусном этаноле и окрашенных по Романовскому. Рассчитывали индексы адаптации и стресса. Использовали робастное статистическое оценивание и методы описательной статистики. Для сравнения групп использован критерий Краскела – Уоллиса. Апостериорные сравнения проводили методом Данна. В большинстве случаев распределение данных было отличным от нормального. Средние величины оцениваемых показателей гемограммы у всех коров варьировали в пределах физиологической нормы, общепринятой для крупного рогатого скота. Наиболее высоким адаптационным потенциалом характеризовались коровы голштинской породы: количество лейкоцитов составило 12,1 × 10^9/л, относительное число сегментоядерных нейтрофилов – 37,5%, индекс адаптации – 3,17, индекс стресса – 0,29. Выявлены межпородные различия по всем оцениваемым показателям, за исключением палочкоядерных нейтрофилов ($p < 0,05$). Наибольшая сила влияния фактора породы установлена для эозинофилов, эритроцитов и тромбоцитов; наименьшая – для моноцитов. Величина η^2 для данных показателей составила 0,43; 0,29; 0,29 и 0,44 соответственно. Полученные данные отражают дифференцированный вклад генетических факторов в разные гематологические показатели.

Ключевые слова: гематологический статус, крупный рогатый скот, порода, референсный интервал, лейкоцитарные индексы

Interbreeding differences in hematologic parameters of cows in Western Siberia

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The paper presents the results of the analysis of the content and variability of hematological status indicators in cows of different breeds bred in Western Siberia. Data on the assessment of differences in the absolute number of leukocytes, erythrocytes, platelets, hemoglobin concentration, relative number of leukocyte cells, stress and adaptation indices between animals of Holstein, Black-and-White, Red Steppe breeds are demonstrated. The peripheral blood of healthy cows of the 2nd lactation was examined. Hematological parameters were measured using the PCT 90Vet automatic hematology analyzer. The leukocyte formula was calculated in blood smears fixed in 950 ethanol and stained according to Romanovsky. Adaptation and stress indices were calculated. Robust statistical estimation and descriptive statistics methods were used. The Kruskal-Wallis criterion was used to compare the groups. Post hoc comparisons were carried out using the Dunn's method. In most cases, the data distribution was different from normal. The average values of the estimated hemogram parameters in all cows varied within the physiological norm generally accepted for cattle. Holstein cows were characterized by the highest adaptive potential: the number of leukocytes was 12.1 × 10^9/l, the relative number of

segmented neutrophils was 37.5%, the adaptation index was 3.17, the stress index was 0.29. Interbreed differences were revealed in all evaluated indicators with the exception of band neutrophils ($p < 0.05$). The greatest influence of the breed factor has been established for eosinophils, erythrocytes and platelets; the lowest for monocytes. The value of n^2 for these indicators was 0.43; 0.29; 0.29; 0.44, respectively. The data obtained reflect the differentiated contribution of genetic factors to different hematological parameters.

Keywords: hematological status, cattle, breed, reference interval, leukocytal index

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INTRODUCTION

Study of the hematological profile of farm animals is of key importance for characterizing their physiological status, adaptive potential, and assessing their welfare and health status. These studies are important for optimizing feeding, identifying potential problems at various reproductive stages, which ultimately contributes to production efficiency[1, 2] [1–6].

Blood parameters, formed under the influence of many genes, their interaction and the environment, are characterized by a fairly high phenotypic variability. Hematological status of the animals is associated with many paratypic predictors[3, 4] [7, 8].

In this case, the homeostasis of hematological parameters, including the number, volume of blood cells, biological activity is strictly regulated within physiological limits, and the deviations are associated with the development of pathologies (anemia, erythrocytosis, etc.) and non-hematological diseases [9–11]. Such variations in phenotypes are caused not only by environmental but also by genetic factors [12–15]. When studying hematological and biochemical parameters in large families, it was shown that blood parameters are highly heritable. Genetic effects explained an average of 40% of the differences in 38 blood parameters [16]. As early as 1985, twin studies demonstrated that from 50 to 90% of interindividual differences in the number of leukocytes, erythrocytes and platelets are due to the genetic component[5].

Recent studies have identified genetic loci associated with mean white blood cell and platelet counts in different ethnic groups, suggesting a genetic basis for differences [17, 18].

Studies of the influence of genetic predictors on blood parameters in cattle are limited. Recently, studies on beef cattle have demonstrated different heredity and genetic correlations of blood parameters between herds with different health conditions [19], works devoted to the comparison of hematological and biochemical profiles of the Brahman and Yunling cattle [20], Hereford

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bulls of different genetic groups [21]. Therefore, the study of hematological parameters of different cattle breeds is important for understanding the contribution of genetic factors to the value of blood parameters.

The purpose of the research is to assess the content and variability of hematological parameters of cows of different breeds raised in Western Siberia. To do this, it is necessary to calculate average values in population samples of cows of the Black-and-White, Holstein and Red Steppe breeds, study the nature of the variation and consider the influence of the breed factor on the hematological profiles.

MATERIAL AND METHODS

The studies were conducted on the cows of three breeds of the 2nd lactation, raised in the conditions of industrial livestock complexes of Western Siberia: Holstein (Kemerovo Region), Black-and-White (Novosibirsk Region) and Red Steppe (Altai Territory). The number of cows in the samples was 51, 57, 53, respectively. The experimental groups included healthy animals that were kept in conditions corresponding to the "Veterinary rules for keeping cattle for the purpose of its reproduction, cultivation and sale", determined by order No. 622 of the Ministry of Agriculture of the Russian Federation dated 21.10. 20.

Blood was collected in the morning hours before feeding the animals from the tail vein (v. coccygea), which is the least stressful method compared to collection from the jugular vein. Blood was collected in vacuum tubes with the anticoagulant dipotassium salt of ethylenediaminetetraacetate (K2EDTA) applied to the walls in the form of microparticles (with a purple cap). The vein and surrounding soft tissues were punctured in one go with a sterile disposable medical needle designed for vacuum blood collection systems. Blood samples were delivered to the Laboratory of Biochemistry and Ecological Genetics of the Department of Veterinary Genetics and Biotechnology in a thermobox with refrigerants at a temperature of +2…+4 °C, avoiding increased mechanical impact on the samples. Hematological parameters were measured using an automatic hematological analyzer 3 dif for 18 parameters PCT 90Vet (USA). The leukocyte formula was counted under a microscope at a magnification of 10 × 90, in blood smears fixed in 95-degree ethanol and stained according to Romanovsky. The stress index was determined by the ratio of segmented neutrophils to lymphocytes in the leukocyte formula. The adaptation index was calculated as the inverse ratio of lymphocytes to segmented neutrophils.

The distributions of the obtained data were checked for normality using the Anderson-Darling (AD) and/or Shapiro-Wilk (SW) tests. Tukey's method was used to determine the outliers. The median, quartiles, and limits were used to present the data. Intergroup comparisons were performed using the Kruskal-Wallis test, and post hoc analysis was performed using the Dunn's method with the Holm's correction. The effect size was assessed using $\eta^2 = (H–k+1)/(n–k)$, where H – Kruskal-Wallis statistics, k – the number of comparison groups.

Calculations were performed in the programs Microsoft Office Excel 16.0 and R x 64 3.6.2.

RESULTS AND DISCUSSION

The level of all studied hematological parameters in cows was within the generally accepted physiological norm, which indicates the absence of diseases in this population group (see Table 1). This also allows comparisons to be made between breeds without concerns about the influence of pathological processes on blood parameters. In most cases, the levels of leukocytes are taken as normative in cattle – 5,0–16,0 × 10^9/l; erythrocytes – 5,5–8,5 × 10^{12}/l; platelets – 270,0–500,0 × 10^9/l; hemoglobin – 80–139 g/l; eosinophils – 2–20%, band neutrophils – 1–4%, segmentonuclear neutrophils – 15–45%; lymphocytes – 45–75%, monocytes – 2–7%[6, 7] [3, 6].

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Табл. 1. Гематологические показатели коров разных пород Западной Сибири
Table 1. Hematological parameters of cows of different breeds in Western Siberia

Indicator	Me	Lim		Q1	Q3	IQR
1	2	3	4	5	6	7
Holstein breed						
Absolute leukocyte count, $\times 10^9$/l	12,1	1,8–41,7		7,9	23,6	15.7
Absolute erythrocyte count, $\times 10^{12}$/l	5,45	3,82–7,5		5,02	6,05	1.03
Platelets, $\times 10^9$/l	197,0	52–286,0		134,0	242,0	108,0
Hemoglobin, g/l	87,0	67–105,0		81,5	9,01	9,5
Leukogram						
Granulocytes: eosinophils, %	1,0	0,0–4,0		0,0	2,0	2,0
Stab neutrophils, %	1,0	0,0–2,0		0,0	1,0	1,0
Segmented neutrophils, %	22,5	10,0–44		19,0	29,0	10,0
Agranulocytes: lymphocytes, %	73,5	47,0–84,0		64,5	76,0	11,5
monocytes, %	2,0	0,0–6,0		1,0	4,0	3,0
Leukocytal indexes of:						
adaptation	3,17	0,48– 6,07		2.13	3.89	1,86
stress	0,29	0,12–0,79		0,25	0,43	0,176
Black-and-White breed						
Absolute leukocyte count,$\times 10^9$/l	9,85	3,9–16.1		8,1	11,25	3,15
Absolute red blood cell count, $\times 10^{12}$/l	6,68	5,27–7,86		6,24	7,09	0,85
Absolute platelet count, $\times 10^9$/l	328,5	94,0–596		257	397	140
Hemoglobin concentration, g/l	91	70,0–109		85,5	96	10,5
Leukogram						
Granulocytes: eosinophils, %	10	0,0–27,0		7,0	15,0	9,0
Stab neutrophils, %	0,0	0,0–2,0		0,0	1,0	1,0
Segmented neutrophils, %	22	5,0–64,0		15	36	21
Agranulocytes: lymphocytes, %	59,5	17,0–82,0		48	70	22
monocytes, %	1,0	0,0–6,0		1,0	3,0	2,0
Leukocytal indexes of:						
adaptation	2,71	0,09–8,75		1,26	4,26	3,0
stress	0,34	0,06–1,59		0,23	0,7	0,47
Red Steppe breed						
Absolute leukocyte count, $\times 10^9$/l	8,2	5–15,6		6,8	9,3	2,5
Absolute red blood cell count, $\times 10^{12}$/l	5,91	3,37–7,49		1,98	6,78	1,34
Absolute platelet count, $\times 10^9$/l	360	341 – 574		353	372	19
Hemoglobin concentration, g /l	98	73–129		90	107	17
Leukogram						
Granulocytes: eosinophils, %	3,0	0,0–11,0		1,0	4,0	3,0
Stab neutrophils, %	1,0	0,0–5,0		0,0	2,0	2,0
Segmented neutrophils, %	30	5,0–55,0		22,5	40	17,5

Окончание табл. 1

1	2	3	4	5	6	7
Agranulocytes: lymphocytes, %	59	37,0–90,0	50		69,5	19,5
monocytes, %	2,0	0,0–7,0	1,0		4,0	3,0
Leukocytal indexes of:						
adaptation	2,14	0,7– 5,27	1,25		2,85	1,64
stress	0,47	0,06–1,45	0,32		0,79	0,46

Note. Me – median value; Q1 – first quartile, Q3 – third quartile, IQR – interquartile range.

Despite the fact that the number of animals in the breed groups was more than 50, and outliers were excluded, in a number of cases the distribution of traits did not correspond to the normal (AD *p-value* < 0,05, SWp < 0,05), therefore the data are presented with robust statistics.

Additional information about the state of the body, including stress conditions, impaired adaptive responses and subclinical manifestations of diseases, can be provided by integral leukocyte indices. Adaptation and stress indices based on the ratio of neutrophils and lymphocytes reflect two aspects of the immune system: the innate immune response, mainly provided by neutrophils, and adaptive immunity supported by lymphocytes [9, 22].

Neutrophilic leukocytes play a key role in the body's primary immune response against infections, using a variety of molecular effects such as phagocytosis, chemotaxis, and the release of reactive oxygen species. As regulators of innate immunity, neutrophils activate and coordinate other cells of the immune system by producing a variety of cytokines and chemokines [23]. Therefore, newborn calves, in whose blood there is the largest number of neutrophils, have the highest values of the stress index (see footnote 7). During ontogenesis, the blood of adult cows acquires a lymphocytic type, the adaptation index increases. Lymphocytes, including various populations, are responsible for adaptive immunity, providing an antigen-specific response, participate in the host's reaction to viruses and allergens. The highest values of the adaptation index and the lowest stress index values are established for the Holstein cows, reflecting the high adaptive potential of this breed.

Since the distribution of the parameters in many cases was not normal and data transformation did not give an effect, interbreed differences were analyzed using the Kruskal-Wallis test (see Table 2). For all the hemogram parameters evaluated, except for band neutrophils, the influence of the breed factor was established. The greatest influence was characteristic of eosinophils, erythrocytes and platelets; the least – for monocytes, which is consistent with the literature data [17].

The use of the Dunn's test for pairwise post hoc comparisons allows us to identify the differences in hematological parameters between different breeds of cows (see Table 3).

Pairwise comparisons showed that cows of the Red Steppe breed were characterized by a lower number of leukocytes compared to the animals of the Holstein and Black-and-White breeds, while at the same time they had a higher level of platelets, hemoglobin, and segmented neutrophils (*p-value* < 0,05). Holstein cows had the highest level of segmented neutrophils. The breeds were ranked in descending order by the number of erythrocytes: Black-and-White – Red Steppe – Holstein ($p < 0,05$).

Hematological characteristics of farm animals demonstrate breed-specific features, taking into account the influence of sex, age and physiological status. Studies to assess interbreed differences in hemograms will be important for assessing the contribution of genetic factors to the phenotypic dispersion of hematological parameters and understanding the features of the formation of the cellular composition of the blood both in health and in diseases.

Табл. 2. Влияние фактора породы на уровень показателей гемограммы коров
Table 2. Influence of the breed factor on the level of cow hemogram parameters

Indicator	*H*	*p*	η2
Absolute leukocyte count, $\times 10^9$/l	20,15	0,0000421*	0,098
Absolute red blood cell count, $\times 10^{12}$/l	55,97	7,01e-13*	0,29
Absolute platelet count, $\times 10^9$/l	43,54	3,509e-10 *	0,29
Hemoglobin concentration, g/l	20,37	0,00003769*	0,11
Eosinophils, %	90,58	0,01e-13*	0,43
Stab neutrophils, %	5,248	0,07248	0,016
Segmented neutrophils, %	17,9016	0,0001296*	0,079
Lymphocytes, %	27,4523	0,000001093*	0,13
Monocytes, %	10,6543	0,004858 *	0,044
Adaptation index	14,9015	0,000581*	0,065
Stress index	17,5848	0,0001519*	0,079

Note. df = 2; η2 – effect size indicator.
Here and in Table 3. * p < 0,05 – statistically significant differences between the groups.

Табл. 3. Межпородные сравнения гематологических показателей коров
Table 3. Interbreeding comparisons of hematological parameters of cows

Indicator	Holstein – Black-and-White	Holstein – Red Steppe	Black-and-White – Red Steppe
leucocytes, $\times 10^9$/l			
Z(p)	1,9587 (0,8268)	4,4551 (0,000008385)*	2,8268 (0,004701) *
Red blood cells, $\times 10^{12}$/l			
Z(p)	7.38 (1.537e-13)*	2,98 (0,0028)*	4,19 (0,00002733)*
Platelets, $\times 10^9$/l			
Z(p)	5,23 (1,688e-7)*	6,59 (4,212э-11)*	2,39 (0,01667)*
Hemoglobin, g/l			
Z(p)	1,792 (0,07313)	3,9733 (0,00007087)*	3,4082 (0,000654)*
Eosinophils,%			
Z(p)	8,8952 (0,00 7e-13)*	2,1959 (0,0281)*	6,8713 (6,362e-12)*
Segmented neutrophils,%			
Z(p)	0,1053 (0,9161)	3,4229 (0,0006197)*	3,8637 (0,0001117)*
Lymphocytes,%			
Z(p)	4,8474(0,000001251)*	4,3933 (0,00001116)*	0,2725 (0,7853)
Monocytes,%			
Z(p)	1,9986 (0,04565)*	1,0315 (0,3023)	3,1885 (0,00143)*
Adaptation index			
Z(p)	1,2439 (0,2135)	3,7276 (0,0001933)*	2,7734 (0,005548)*
Stress Index			
Z(p)	1,282 (0,1999)	4,006 (0,00006175)*	3,0482 (0,002302)*

CONCLUSIONS

1. The average values of hemogram indices in cows of the Holstein, Black-and-White and Red Steppe breeds at the age of the 2nd lactation, raised in Western Siberia, varied within the physiological norm. The highest adaptive potential was characterized by cows of the Holstein breed: the number of leukocytes was 12,1 × 10^9/l, relative number of segmented neutrophils – 37,5%, adaptation index – 3,17, stress index – 0,29.

2. Interbreed differences in the number of leukocytes, erythrocytes, platelets, hemoglobin concentration, relative number of cells of the leukocyte formula, with the exception of band neutrophils, stress and adaptation indices were revealed. The greatest influence of the breed factor was established for eosinophils, erythrocytes and platelets; the least – for monocytes.

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Влияние биоактивированного зерна пшеницы, обогащенного селенитом натрия, на продуктивные показатели перепелов

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Представлены результаты исследований эффективности использования зерна пшеницы, биоактивированного двумя способами, в составе рационов птиц. Исследования проведены на перепелах японской селекции от рождения до 153-дневного возраста. В однодневном возрасте сформированы три группы цыплят по 70 гол. в каждой. Контрольная группа получала основной рацион (ОР). Первая опытная группа получала ОР + биоактивированное водой зерно пшеницы (20% от массы нативного зерна в корме). Вторая опытная группа получала ОР + биоактивированное с селенитом натрия (селен 25 мг/л воды) зерно пшеницы (20% от массы нативного зерна в корме). Установлено, что по сравнению с нативным зерном в рационе контрольной группы при биоактивации пшеницы комбикорма 1-й и 2-й опытных групп в зерне повышается сахар от 4,99 до 8,17 и 7,20% со снижением крахмала с 76,23 до 72,49 и 71,95% и увеличением уровня жира с 1,35 до 1,51 и 1,74% соответственно. Включение в ОР перепелов биоактивированного зерна в 1-й опытной группе способствует повышению сохранности молодняка перепелов на 4,3%, во 2-й опытной группе – на 7,1% по сравнению с контрольной группой; живой массы за 60 дней опыта – на 6,0 и 7,2% ($P \geq 0,99$); среднесуточного прироста – на 6,5 и 7,4% ($P \geq 0,99$); снижению затрат корма от 7,0 до 8,5%; убойного выхода – на 2,0 и 4,2%; повышению содержания в мышечной массе лизина от 0,73 ($P \geq 0,99$) до 0,79% ($P \geq 0,99$). Установлено положительное влияние биоактивированного зерна в составе комбикорма на показатели воспроизводства: несушки 1-й и 2-й опытных групп начали проброс яиц на 3 дня раньше, чем в контроле. Больше яиц за учетный период получено от несушек перепелов 2-й опытной группы – 72,48 шт., что превышает показатели контрольной группы на 8,7% и 1-й опытной – на 7,8%. По яичной массе куры-несушки 2-й опытной группы превысили контроль на 8,2%, по условному экономическому эффекту при производстве товарных яиц – на 14,0%.

Ключевые слова: зерно пшеницы, биоактивация, селен, молодняк перепелов, перепела-самцы и несушки, продуктивность

Effect of bioactivated wheat grain enriched with sodium selenite on productive performance of quails

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The results of the studies on the effectiveness of using wheat grain, bioactivated in two ways, in the diet of quails are presented. The studies were carried out on the quails of Japanese selection from birth to 153 days of age. At one day of age, three groups of chickens of 70 heads each were formed. The control group received the basic diet (BD). The first experimental group received BD + bioactivated with water wheat grain (20% of the weight of native grain in the feed). The second experimental group received BD + bioactivated wheat grain with sodium selenite (selenium 25 mg/l of water) (20% from the mass of native grain in the feed). It was found that during bioactivation in wheat grain of experimental groups I and II, sugar increases compared to native grain in the control group from 4.99% to 8.17 and 7.20%, with a decrease in starch from 76.23 to 72.49 and 71.95%, increasing the fat level from 1.35 to 1.51 and 1.74%, respectively. The inclusion of bioactivated grain in the basic diet of quails in the first experimental group helps to increase the viability of young quails by 4.3%, in the second experimental group – by 7.1% compared to the control group; live weight for 60 days of experiment by 6.0 and 7.2% ($P \geq 0.99$), average daily gain – by 6.5 and 7.4% ($P \geq 0.99$), reduction in feed

costs from 7.0 to 8.5%, slaughter yield – by 2.0 and 4.2%, an increase of lysine content in muscle mass from 0.73 ($P \geq 0.99$) to 0.79% ($P \geq 0.99$). A positive effect of the activated grain in the composition of feed on the reproductive indicators was established: laying hens of the I and II experimental groups began laying eggs 3 days earlier than in the control. More eggs during the record period were obtained from laying quails of the II experimental group – 72.48 pieces, which exceeds the indicators of the control group by 8.7% and the I experimental group by 7.8%. In terms of egg mass, laying hens of the second experimental group exceeded the control by 8.2%, and in terms of the conditional economic effect in the production of marketable eggs – by 14.0%.

Keywords: wheat grain, bioactivation, selenium, young quails, male quails and laying hens, productivity

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INTRODUCTION

Grain feed as a source of carbohydrates in the compound feed recipes makes up to 80%. The disadvantage of grain components is that along with starch they contain non-starch polysaccharides, which create the problem of increased viscosity of the contents of the gastrointestinal tract, negatively affect the absorption of feed and physiological indicators and productivity of poultry [1].

An accessible and inexpensive way to increase the completeness of animal diets is the use of bioactivated grain, known for many millennia. The basis of biological activation is the targeted action on the enzymatic complex of grain by moistening at a comfortable temperature and with air access[1] [2].

Bioactivation of feed grain should last no more than 24 hours, when the grains are still free of mold, fungi and have flowability [3]. The grain should be freshly harvested, without impurities, not infected with fungi, with good germination and without mechanical damage.

The grain is a living organism that is at rest, in which a slow metabolism takes place, supporting the life of the germ cell[2].

Dry cereal seeds contain up to 15% moisture. When humidity increases, free water appears, which sharply increases the intensity of respiration and other metabolic processes. Under the influence of free water, the permeability of cell walls improves, which ensures the transition of enzymes and nutrients into the solution and their migration to the embryo.

As a result of enzyme activation, biochemical processes in grain are accelerated. The effect of temperature on grain promotes expansion of capillaries of the shells and acceleration of water penetration, which catalyzes some positive phenomena that cause structural and biochemical changes in grain. The levels of cellulose and hemicelluloses decrease, but the amount of soluble pentosans increases, hydrolysis of high-molecular biopolymers occurs, and biologically active compounds are formed, providing an increase in the nutritional value of sprouted grain[3] [4].

[1] *Mirontseva A.A., Tsed E.A., Volkova S.V.* Optimization of water-heat treatment modes for batches of bioactivated rye and triticale grains in the production of food ethyl alcohol // Collection of the writings of the VIII International scientific and practical conference "Innovative technologies in the food industry: science, education, production". November 30, 2022, Voronezh, pp. 160–163.

[2] *Veselova A.Yu.* Intensification of preliminary preparation of cereal crops in the context of developing new technology // Bulletin NGIEI, 2011, vol. 2, N 6 (7), pp. 27–37.

[3] *Zenkova M.L., Nazarova O.O.* A new type of canned products from grain raw materials // Proceedings of the Tavria State Agrotechnological University, 2012, vol. 12, N 4, pp. 97–103.

The use of bioactivated grain in poultry farming helps to increase egg production and normalize the microflora of the gastrointestinal tract in hens and broiler chickens, has a positive effect on the height of the ileum villi in broiler chickens, which ultimately improves feed conversion and live weight gain[4, 5] [5]. A positive effect of bioactivated wheat grain on the reproductive capacity of meat chickens has also been revealed[6].

Many enzymes are activated in sprouted grain, so expensive enzyme preparations can be excluded from the animals' diet and animal productivity can be increased. In addition, the feed obtained in this way is cheaper than compound feed[7].

There are several methods for obtaining bioactivated cereal grain: using ultraviolet radiation, using ultrasound treatment, adding organic acids to steeping water, using non-thermal technology (cold plasma), etc.[8, 9] [6, 7].

Among the substances that play a significant role in poultry nutrition, an important place is given to microelements, including selenium, which is necessary for growth, reproduction, hematopoiesis and endocrine gland functions, regulation of metabolism, permeability of cell membranes, protective reactions of the body, for the prevention of white muscle disease, etc.[10]

Plants as the main source of selenium are the first link in the food chain of the element's entry into the animal organism [8]. The need for selenium for higher plants has not been sufficiently studied at present. However, it has been proven that selenium is a controlling element in the influence of protein metabolism in plants, the activity of enzymes and non-enzymatic antioxidants, such as flavonoids, etc.[11] [9].

The absorption of selenium by plants is influenced by many factors: concentration, chemical form of the element, pH of the environment, etc. In practice, sodium selenite is most often used, which is a water-soluble and generally available pool of Se for grain, which is converted into organic selenoprotein [10, 11]. It has been proven that selenium from sodium selenite accelerates the rate of germination of wheat seeds compared to the control (without selenium treatment), since, according to a number of authors, there is a similarity between selenite and wheat grains[12, 13].

Recommended rates of sodium selenite addition to aqueous solution when soaking seeds are from 0.001 to 0.005% of the pure element[14] [12].

The purpose of the research is to evaluate the effectiveness of using bioactivated wheat grain in two ways as part of quail diets at different stages of postembryonic development.

[4]*Kassamedinov A.I., Razumovskaya R.G.* The use of sprouted grain in the diet of birds and its importance for the microflora of the gastrointestinal tract // Vestnik of Astrakhan State Technical University, 2011, N 1 (51), pp. 2–27.

[5]*Afsharmanesh M., Paghaleh A.S., Kheirandish R.* Effects of sprouted and nonsprouted wheat and barley with and without enzyme on intestinal morphometry of broiler chickens // Comparative Clinical Pathology, 2012, N 22 (5). DOI: 10.1007/s00580-012-1517-3.

[6]*Charyev A.B., Gadiev R.R.* Efficiency of using sprouted barley grain in the diet of meat chickens of the parent flock // Izvestia Orenburg State Agrarian University, 2015, N 1 (51), pp. 119–121.

[7]*Egorov S.V., Meged S.S., Fomin S.M.* Unconventional methods of increasing the completeness of feeding suckling lambs // Collection of scientific papers / RAAS, Siberian Branch, SibNIPTIZH. Novosibirsk, 2002, pp. 121–127.

[8]*Veselova A.Yu.* Intensification of preliminary preparation of cereal crops in the context of developing new technology // Bulletin NGIEI, 2011, vol. 2, N 6 (7), pp. 27–37.

[9]*Vorobyov D.A., Zabudskiy A.I., Algazin D.N.* Development of a grain bioactivation unit for use in feeding farm animals // Bulletin of the Omsk State Agrarian University, 2017, N 4 (28), pp. 200–204.

[10]*Egorov I.A., Andrianova E.N., Petrosyan A.B., Manukyan A.V., Papazyan T.T., Prisyazhnaya L.M., Rozanov B.L., Egorova T.V., Yudin S.M., Sokolov S.M., Panin A.I.* Organic forms of micronutrients in farm poultry feed. Sergiev Posad, 2010, 44 p.

[11]*Whanger P.D.* Seleno compounds in plants and animals and their biological significance // Journal of the American College of Nutrition, 2002, vol. 21, pp. 223–232. DOI: 10.1080/07315724.2002.10719214.

[12]*Kulagina Yu.M., Golovatskaya I.F.* Effect of sodium selenite on the growth and development of wheat plants depending on the method of treatment // Tomsk State University Journal. Biology, 2011, N 2 (14), pp. 56–64.

[13]*Ali F., Peng Q., Wang D., Cui Z., Huang J., Fu D., Liang D.* Effects of selenite and selenate application on distribution and transformation of selenium fractions in soil and its bioavailability for wheat (*Triticum aestivum* L.) // Environmental Science and Pollution Research International, 2017, vol. 24 (9), pp. 8315–8325. DOI: 10.1007/s11356-017-8512-9.

[14]*Shchukin V.B., Gromov A.A., Shchukina N.V.* Selenium as an exogenous stimulating factor in the initial period of growth and development of winter wheat plants // Izvestia Orenburg State Agrarian University, 2005, N 3 (7), pp. 107–110.

The objectives of the study included determining the effect of bioactivated grain in compound feed in two ways on the survival rate of quails, growth rate, feed costs, meat productivity, meat quality, egg production, and efficiency.

MATERIAL AND METHODS

The research was conducted in two stages

First stage

Before bioactivation, the grain germination rate was determined according to GOST 12038–84, amounting to 95–96% in three replicates. The grain of soft wheat of the Obskaya variety was taken for the research.

Bioactivation of grain was carried out according to recommendations in the following way: a sample of native wheat grain was washed twice in tap water, and the floating debris was separated[15].

The Teflon-coated baking sheets were covered with two layers of cloth, then the grain was poured in and filled with water according to the experimental scheme in a ratio of 1:2 until completely covered. After 2 hours, the liquid was drained, the grain was covered with cloth (in two layers) moistened with water. During the next 20 hours, the grain was stirred and sprayed 2–3 times. Then it was distributed in a layer no more than 2 cm high on a surface covered with cellophane and cloth. At room temperature, the wet grain was dried for two days until the humidity level of 11,32–11,36%.

After drying, the bioactivated grain was crushed in a Farmer IGE-05 grain crusher to a grind of at least 1–2 mm and then according to the experimental design.

In the biochemical laboratory of the Siberian Research and Design and Technological Institute of Animal Husbandry of the Siberian Federal Scientific Centre of Agro-BioTechnologies of the Russian Academy of Sciences (SibRDTI-AH SFSCA RAS), the chemical composition of wheat grain in its native state and after drying of bioactivated grain, starter and finishing compound feed for quails, and the concentration of selenium in the sodium selenite preparation were determined using generally accepted methods.

Second stage

A scientific and economic experiment lasting 153 days was conducted according to the generally accepted methodology[16] in 2023 in the vivarium conditions of the physiological yard of the SFSCA RAS according to the following scheme (see Table 1).

To study the task, three groups of one-day-old Japanese quails were formed, 70 heads each, taking into account the requirements of the generally accepted poultry farming methods. The conditions of keeping and feeding corresponded to zootechnical standards.

The quails of the control group received the basic diet (BD). For the quails of the 1st experimental group, 20% of native wheat was replaced by wheat grain bioactivated with tap water at

Табл. 1. Схема опыта

Table 1. Experiment scheme

Group	Number of heads	Feeding conditions
Control	70	BD*
Experimental		
1st	70	BD + bioactivated wheat grain (20% of the native grain mass in the compound feed composition)
2nd	70	BD + bioactivated with sodium selenite (selenium 25 mg/l of water) wheat grain (20% of the native grain mass in the compound feed)

*BD – basic diet, balanced according to the VNIIITIP standards (2011).

[15]*Koneva M.S.* Development of technology and evaluation of consumer properties of smoothies enriched with products from sprouted wheat grain: Candidate's thesis in technical sciences / M.S. Koneva. Krasnodar, 2017, 175 p.

[16]Methodology for conducting scientific and industrial research on feeding agricultural poultry // Russian Academy of Agricultural Sciences; Scientific and Technical Center "Plemptitsa"; State Scientific Institution VNITIP; under the general editorship of V.I. Fisinin and Sh.A. Imangulov. Sergiev Posad, 2000, 33 p.

room temperature. The quails of the 2nd experimental group received in addition to the basic diet 20% of native wheat grain, the same amount of grain bioactivated with tap water at room temperature, which included sodium selenite (25 mg/l of water by pure element) during the period of rearing young animals (1–60 days) and productive use of quail laying hens (61–153 days).

The feed consumed, the condition and safety of the quails were recorded daily; for quail laying hens, the start date of egg-laying and the counting of eggs. Quails were weighed individually in groups at the age of one day, then at the age of 30 and 60 days.

Slaughter of the cockerels was carried out at the age of 60 days, 5 heads from each group for studying the biochemical composition of muscle mass in the biochemical laboratory of the SibRDTIAH SFSCA RAS and the indicators of slaughter yield of the carcasses.

From the beginning of egg laying, daily egg counts were made from layers of all groups. The thickness of the shell was measured monthly with a micrometer.

Economic efficiency is calculated based on the difference in costs for the purchase of feed and feed additives per unit of output, taking into account the selling price.

The digital material obtained during the experiment was processed by the method of variation statistics on a personal computer using Microsoft Excel software using the criterion of the reliability of the difference according to the Student-Fisher method.

RESULTS AND DISCUSSION

First stage

After 1 day of soaking, the height of the wheat grain sprouts in the main mass was from 1 to 3 mm in the 1st experimental group, from 1 to 4 mm in the 2nd experimental group, an insignificant part of the sprouts reached the length of the roots, respectively of 5 and 6–7 mm.

Study of the dried and ground grain after bioactivation showed, in comparison with the native grain, an increase in sugars from 4.99 to 8.17 and 7.20%, a decrease in starch from 76.23 to 72.49 and 71.95%, an increase in fat levels from 1.35 to 1.51 and 1.74% in the samples of experimental groups (see Table 2). In terms of the content of exchange energy, there was practically no difference between the samples (14.01–14.05 MJ ME).

In the starter compound feed (age of the quails from the 1st to the 30th day) in comparison with the control group, an increase in the fat level in the 1st and 2nd experimental groups was noted from 3.15 to 4.66 and 3.67%, fiber from 5.09 to 7.50 and 5.28%, lysine from 0.57 to 0.70 and 1.00%, a decrease in starch from 28.30 to 26.00 and 26.20%. The sugar level, according to the biochemical laboratory of the SibRDTIAH SFSCA RAS, was increased only in the 2nd experimental group in comparison with the control by 3.20% (see Table 3).

In the finishing compound feed (from the 31st to the 60th day and further) in the 1st experimental group, the fat content was 3.08%, which exceeded the control by 0.76%. The sugar level in the 2nd experimental group, compared to the control group, increased from 12.90 to 16.10%, starch in both experimental groups decreased

Табл. 2. Химический состав зерна пшеницы, %
Table 2. Chemical composition of wheat grain, %

Indicator	Native grain	Bioactivated grain	
		with water	with water with sodium selenite
Humidity	12,57	11,36	11,32
Dry matter	87,43	88,64	88,68
Crude protein	10,87	11,02	10,88
Crude fat	1,35	1,51	1,74
Fiber	2,11	2,51	2,17
Sugars	4,99	8,17	7,20
Starch	76,23	72,49	71,95
NFES	71,55	72,14	72,42
Lysine	0,275	0,254	0,263
Crude ash	1,55	1,46	1,47
Calcium	0,150	0,153	0,150
Phosphorus	0,346	0,364	0,343
EFU by crude DF	1,40	1,40	1,40
ME по сырым DF, MJ	14,05	14,04	14,01

from 28.30 to 26.00 and 26.20%, an increase in lysine was noted from 0.53 to 0.70 and 0.60%.

There were also no significant differences in the amount of exchange energy in the feeds of all groups.

Second stage

The studies showed that the inclusion of bioactivated wheat grain in the diets of quails in the 1st and 2nd experimental groups had a positive effect on their growth rate (see Table 4). At 30 days of age, quails in the 2nd experimental group exceeded the control group in live weight by 8.2% ($P \geq 0.999$). At 60 days of age, the live weight growth rate of quails in both experimental groups was higher by 6.0 and 7.2% ($P \geq 0.99$) compared to the control. The average daily gains of chickens during the entire study period in all groups corresponded to the gain in their live weight.

Табл. 3. Химический состав рационов перепелов, %

Table 3. Chemical composition of quail diets, %

Indicator	Group		
	control	experimental	
		1st	2nd
Starter feed			
Humidity	12,68	11,32	12,44
Dry matter	87,32	88,68	87,56
Crude protein	20,61	20,63	20,63
Crude fat	3,15	4,66	3,67
Crude fiber	5,09	7,50	5,28
Sugars	12,90	11,30	16,10
Starch	28,30	26,00	26,20
Crude ash	10,58	9,80	9,92
Lysine	0,57	0,70	1,00
Calcium	2,05	1,98	2,10
Phosphorus	1,10	0,97	1,05
ME, MJ	13,08	13,10	13,23
EFU	1,31	1,31	1,32
Finisher feed			
Humidity	12,30	11,56	11,92
Dry matter	87,70	88,44	88,08
Crude protein	19,47	19,58	19,89
Crude fat	3,22	3,98	3,31
Crude fiber	7,66	7,62	7,54
Sugars	11,20	16,70	16,20
Starch	33,50	24,20	26,10
Crude ash	12,75	13,61	14,05
Lysine	0,53	0,70	0,60
Calcium	2,79	2,36	2,57
Phosphorus	1,17	1,19	1,33
ME, MJ	12,39	12,40	12,35
EFU	1,24	1,24	1,24

At the age of 1 month (see Table 5), the young female quails in the 2nd experimental group exceeded the control by 8.0% ($P \geq 0.99$), males – by 8.1% ($P \geq 0.99$). At the age of 2 months, the females of the 1st experimental group were larger by 4.3% ($P \geq 0.95$), the 2nd – by 6.7% ($P \geq 0.95$), males, respectively, by 3.5% ($P \geq 0.95$) and 4.4% ($P \geq 0.95$) compared to the control group.

During the record period of the research, the feed consumption of young quails in the experimental groups was at the same level for the first month, then slightly decreased (by 1.6%) compared to the control group. As a result, in the experimental groups, feed costs for the gain of 1 g of live weight were lower by 7.1 and 8.5% compared to the control.

In the experimental groups, by the age of 2 months, the survival rate of young quails ranged from 92.9 to 95.7% versus 88.6% in the control group, which was 4.3% higher in the 1st group and 7.1% higher in the 2nd group.

At the end of the rearing period, the male quails were slaughtered. The results of the control slaughter of the birds showed that the average pre-slaughter live weight of cockerels in the 1st and 2nd experimental groups was higher than in the control group by 5.0 ($P \geq 0.999$) and 8.7% ($P \geq 0.999$), the weight of the eviscerated carcass – by 8.0 ($P \geq 0.999$) and 13.24% ($P \geq 0.999$). As a result, the slaughter yield of carcasses in the experimental groups was respectively higher by 2.0 and 4.2% compared to the control (see Table 6). The use of modified diets did not affect the weight of the internal organs of quail carcasses.

The biochemical composition of quail muscle tissue mince showed that the inclusion of bioactivated wheat grain with sodium selenite in

Табл. 4. Динамика живой массы молодняка перепелов, затраты корма, в среднем на 1 гол.
Table 4. Dynamics of live weight of young quails, feed costs, on average per 1 head

Indicator	Group		
	control	Experimental	
		1st	2nd
Age, days:	*Live weight, g*		
1	8,47 ± 0, 08	8,40 ± 0,09	8,49 ± 0,08
30	131,00 ± 2,18	136,07 ± 1,82	141,7 9 ± 1,25**
60	194,03 ± 2,26	205,73 ± 2,26**	207,9 5 ± 2,61**
Period, days:	*Average daily gain, g*		
1–30	4,08	4,26	4,44
31–60	2,12 ± 0,07	2,32 ± 0,07*	2,44 ± 0,08**
1–60	3,09 ± 0,04	3,29 ± 004**	3,32 ± 0,04**
Period, days:	*Feed costs, kg*		
1–30	0,635	0,635	0,625
31–60	1,129	1,111	1,111
Feed consumed, total, kg	1,764	1,746	1,736
Period, days:	*Feed costs per 1 g of gain, g*		
1–30	5,18	4,97	4,69
31–60	17,84	15,95	16,79
1–60	9,51	8,84	8,70
Feed costs per 1 g of gain during the experimental period, % of the control group	100,0	92,95	91,48

Here and in tables 5–8, 13.
*$P \geq 0,95$.
**$P \geq 0,99$.
***$P \geq 0,999$.

Табл. 5. Сохранность, живая масса молодняка перепелов, в среднем на 1 гол.
Table 5. Viability, live weight of young of quail, on average per 1 head

Indicator	Group		
	control	experimental	
		1st	2nd
Number	*Quails, total*		
At the beginning of the experiment, heads	70	70	70
At the end of the experiment, heads	62	65	67
Viability, %	88,6	92,9	95,7
Live weight	*Females*		
At 30 days of age, g	132,76 ± 3,17	137,52 ± 2,88	143,35 ± 1,79**
At 60 days of age, g	207,26 ± 3,66	216,09 ± 2,77*	221,17 ± 3,81*
	Males		
At 30 days of age, g	129,24 ± 3,02	134,57 ± 2,42	139,75 ± 1,62**
At 60 days of age, g	186,23 ± 2,05	192,79 ± 1,75*	194,33*

the diet of young quail in the 2nd experimental group contributed to a decrease in muscle tissue moisture by 1.3% ($P \geq 0.99$) with an increase in dry residue by 1.3% ($P \geq 0.99$). Moreover, the mineral part (raw ash) in the experimental groups increased by 16.0 ($P \geq 0.999$) and 10.2% ($P \geq 0.99$) in comparison with the control group, including calcium – by 14.9 ($P \geq 0.99$) and 16.1% ($P \geq 0.99$), phosphorus – by 5.1 ($P \geq 0.95$) and 8.7% ($P \geq 0.999$) (see Table 7).

When studying the amino acid composition of muscle tissue samples, which characterizes the quality of the products, an increase in the level of all essential amino acids, except phenylalanine, was noted in the experimental groups (see Table 8). Thus, the level of lysine was higher in the 1st experimental group by 0.79% ($P \geq 0.99$), in the 2nd – by 0.73% ($P \geq 0.99$), methionine, respectively, by 0.10 ($P \geq 0.95$) and 0.16% ($P \geq 0.999$), threonine – by 0.31 ($P \geq 0.99$) and 0.30% ($P \geq 0.999$), valine – by 0.31 ($P \geq 0.95$) and 0.31% ($P \geq 0.99$), histidine – by 0.18 ($P \geq 0.99$) and 0.16% ($P \geq 0.99$), leucine + isoleucine – by 0.88 ($P \geq 0.99$) and 0.65% ($P \geq 0.99$) compared with the control group.

In terms of replaceable amino acids in samples of minced meat from the quail carcasses of the experimental groups, a reliable increase in the content of alanine, glycine, proline, serine, and tyrosine was noted.

The sum of essential amino acids in the 1st experimental group was 1.5 times higher than the control, in the 2nd experimental group – 1.4 times. The sum of the studied non-essential amino acids was also 1.5 and 1.4 times higher than the control, respectively.

When calculating the economic effect, the costs of feed, feed additives, grain preparation during bioactivation, as well as the cost of selling the obtained products were taken into account.

Табл. 6. Показатели убойного выхода тушек перепелов в 2-месячном возрасте, $n = 5$
Table 6. Indicators of slaughter yield of quail carcasses at 2 months of age, $n = 5$

Indicator	Group		
	control	experimental	
		1st	2nd
Pre-slaughter live weight, g	187,8 ± 1,43	197,2 ± 1,27***	204,2 ± 1,14***
Weight of gutted carcass, g	134,4 ± 47	145,2 ± 1,71***	152,2 ± 2,45***
Slaughter yield, %	71,6 ± 1,01	73,6 ± 0,95	75,8 ± 0,69
Liver weight, g	3,60 ± 0,24	3,40 ± 0,15	3,60 ± 0,15
Stomach weight, g	5,30 ± 0,12	5,00 ± 0,09	5,51 ± 0,18
Heart weight, g	1,90 ± 0,06	1,90 ± 0,06	2,02 ± 0,12

Табл. 7. Биохимический состав фарша мышечной ткани перепелов, при натуральной влажности, $n = 5$, %
Table 7. Biochemical composition of minced quail muscle tissue, at natural humidity, $n = 5$, %

Indicator	Group		
	control	experimental	
		1st	2nd
Humidity	72,89 ± 0,33	73,09 ± 0,37	71,56 ± 0,10**
Dry matter	27,11 ± 0,33	26,91 ± 0,37	28,42 ± 0,19**
Crude protein	20,81 ± 0,08	21,18 ± 0,27	21,49 ± 0,38
Crude fat	5,30 ± 0,34	4,48 ± 0,27	5,65 ± 0,40
Crude ash	0,998 ± 0,043	1,158 ± 0,0126***	1,100 ± 0,015**
Calcium	0,087 ± 0,003	0,100 ± 0,003**	0,101 ± 0,002**
Phosphorus	0,276 ± 0,004	0,290 ± 0,005*	0,300 ± 0,004***

Табл. 8. Аминокислотный состав мышечной ткани перепелов 2-месячного возраста, *n* = 5, %
Table 8. Amino acid composition of muscle tissue of quails 2 months of age, *n* = 5, %

Indicator	Group		
	control	experimental	
		1st	2nd
Essential amino acids			
Arginine	0,97 ± 0,05	1,42 ± 0,15*	0,97 ± 0,14*
Valine	0,66 ± 0,06	1,32 ± 0,05**	0,97 ± 0,04**
Histidine	0,53 ± 0,03	0,69 ± 0,03**	0,67 ± 0,03**
Lysine	1,55 ± 0,13	2,34 ± 0,18**	2,28 ± 0,12**
Leucine + isoleucine	1,85 ± 0,12	2,73 ± 0,26**	2,50 ± 0,14**
Methionine	0,38 ± 0,02	0,48 ± 0,04*	0,54 ± 0,02***
Threonine	0,60 ± 0,04	0,91 ± 0,10**	0,90 ± 0,05***
Phenylalanine	0,51 ± 0,03	0,74 ± 0,10	0,68 ± 0,04
Sum of essential amino acids	7,05	10,28	9,86
Non-essential amino acids			
Alanine	0,97 ± 0,07	1,41 ± 0,16*	1,41 ± 0,08**
Glycine	0,58 ± 0,04	0,90 ± 0,12	0,83 ± 0,05
Proline	0,54 ± 0,03	0,79 ± 0,09*	0,73 ± 0,04**
Serine	0,61 ± 0,04	0,93 ± 0,12**	0,91 ± 0,04***
Tyrosine	0,45 ± 0,02	0,57 ± 0,07*	0,55 ± 0,02**
Sum of non-essential amino acids	3,15	4,60	4,43
Total	10,20	14,88	14,29
Amino-acid index	2,24	2,23	2,23

The difference between these indicators was used to determine this value (see Table 9). As a result, the best indicators of economic efficiency were noted in the experimental groups – exceeding the control group by 46.62 and 71.48%.

The remaining livestock continued to use the same feed in accordance with the experimental design in order to study the egg production of quail laying hens up to 5 months of age.

The hens of the experimental groups started

Табл. 9. Экономический эффект при выращивании молодняка перепелов (при убое в возрасте 60 дней), на 1 гол., *n* = 5
Table 9. Conditional economic effect when raising young quails (at slaughter at the age of 60 days), per 1 head, *n* = 5

Indicator	Group		
	control	experimental	
		1st	2nd
Cost of 1 kg of starter feed, rubles	46,00	46,35	46,79
Cost of 1 kg of finisher compound feed, rubles	38,00	38,35	38, 75
Cost of feed per head, rubles	72,11	72,02	72,78
Including costs for bioactivation of grain, rubles	–	0,60	1,33
Costs of bioactivation of grain, % of feed cost	–	0,84	1,83
Quantity of the products received, kg	0,1344	0,1454	0,1522
Cost of selling 1 kg of quail meat, rubles	650,00	650,00	650,00
Cost of production, rubles	87,36	94,38	98,93
Profit received, rubles	15,25	22,36	26,15
To the control group, %	100,00	146,62	171,48

laying eggs at the age of 47 days, while those of the control group started laying eggs at the age of 50 days. No differences in egg weight were observed between the groups (see Table 10). The largest number of eggs during the accounting period were obtained from the quail hens of the 2nd experimental group (72.48 pcs.), which is 8.7% more than in the control group and 7.8% more than in the 1st experimental group, respectively. In terms of egg weight, the quail hens of the 2nd experimental group exceeded the control by 8.8%, and those of the 1st experimental group by 8.0%.

Feed costs during the egg-laying period in the 1st experimental group were higher by 7.6%, in the 2nd – by 3.1% compared to the control. However, more economical feed consumption for the production of 10 eggs was noted in the layers of the 2nd experimental group – less by 5.3% than in the control, and by 8.9% than in the 1st experimental group.

The thickness of the eggshell of laying quails in the experimental groups was 10 μm or 4.55% ($P \geq 0.95$) greater, indicating a positive effect of bioactivated wheat grain on the quality indicators of eggs. The quality and safety of eggs increase with a thicker shell,

At the end of the studies, 30 eggs in each group were selected and calibrated for weight, color, and shape over 5 days and were placed in incubation to determine the effect of modified diets on the reproductive qualities of quail laying hens. As a result, after 17 days, incubation waste in the control group and the 1st experimental group amounted to 20.0%, in the 2nd experimental group – 16.7% (see Table 11).

Taking into account feed costs, the best indicators of economic efficiency per laying hen of quail were obtained in the 2nd experimental group: they exceeded the control by 13.96%, or 51.05 rubles (see Table 12).

Табл. 11. Результаты инкубации, $n = 30$
Table 11. Incubation results, $n = 30$

Indicator	Group		
	control	1st	2nd
Incubation waste, pcs.	6	6	5
Including: unfertilized, pcs.	4	4	5
frozen embryos, pcs.	2	2	–
Incubation waste, % across the group	20,00	20,00	16,67

Табл. 10. Показатели продуктивности несушек перепелов, в среднем на 1 гол.
Table 10. Productivity indicators of quail laying hens, on average per 1 head

Indicator	Group		
	control	experimental	
		1st	2nd
Period	*Weight of quail laying eggs, g*		
Egg laying	10,08 ± 0,31	10,21 ± 0,31	10,31 ± 0,14
Output at 50.0% productivity	12,07 ± 0,08	11,92 ± 0,08	11,95 ± 0,07
At the end of the study	12,28 ± 0,10	12,28 ± 0,10	12,20 ± 0,08
On average	11,48	11,47	11,49
	Productivity indicators		
Total number of eggs received, pcs.	66,66	67,25	72,48
Egg mass, g	765,26	771,36	832,80
Egg mass, % from the control	100,0	100,80	108,83
	Feed costs, kg		
Total for the egg-laying period	2,89	3,11	2,98
For the production of 10 eggs	0,434	0,462	0,411
± from the control group	–	+0,028	–0,023
From the control group, %	100,00	106,45	94,70

Табл. 12. Экономическая эффективность производства яиц перепелов за опытный период, в среднем на одну несушку
Table 12. Economic efficiency of quail egg production during the trial period, on average per one hen

Indicator	Group		
	control	experimental	
		1st	2nd
Cost of 1 kg of finisher compound feed, rubles	38,00	38,35	30,75
Cost of feed per head, rubles	109,82	119,27	115,48
Including costs for bioactivation of grain, rubles	–	1,00	2,11
Number of eggs obtained, pcs.	66,66	67,25	72,48
Selling price of 10 eggs, rubles	97,50	97,50	97,50
Cost of obtained food eggs, rubles	649,94	655,69	706,68
Profit received, rubles	540,12	536,42	591,20
Percentage from the control group	100,00	99,31	113,96

Based on the results of blood tests, it was established that homeostasis indicators in all groups were within the physiological norm (see Table 13).

When studying the protein composition of quail blood serum in samples from the 2nd experimental group, an increase in protein levels by 24.5% ($P \geq 0.999$), globulins by 41.9% ($P \geq 0.999$) was noted with a tendency to increase the amount of albumin by 4.8% ($P = 0.92$) compared to the control. This indicates an increase in the biological status of quails in this group [13].

The uric acid content in the 1st experimental group was reduced by 27.3% ($P \geq 0.999$), in the 2nd experimental group a tendency to decrease by 15.8% was noted ($P = 0.94$), which indicates good metabolism of nitrogen-containing compounds in the body of quails of these groups.

A decrease in the level of bilirubin in the blood of quails of the 1st experimental group by 18.5% ($P \geq 0.95$) may indicate good liver function in the secretion of bile, which is necessary for the activation of digestive enzymes, as well as for participation in the hydrolysis and absorption of the nutrients.

Табл. 13. Биохимический состав сыворотки крови самцов перепелов, $n = 5$
Table 13. Biochemical composition of blood serum of male quails, $n = 5$

Indicator	Group		
	control	experimental	
		1st	2nd
Protein, g/l	33,58 ± 0,68	33,64 ± 0,70	41,81 ± 1,58***
Albumin, g/l	15,64 ± 0,26	15,41 ± 0,34	16,37 ± 0,34
Globulins, g/l	17,94 ± 0,57	18,23 ± 0,42	25,44 ± 1,34***
Uric acid, μmol/l	449,03 ± 23,56	324,80 ± 20,2***	377,88 ± 28,36
Cholesterol, mmol/l	4,40 ± 0,14	4,30 ± 0,21	4,35 ± 0,11
Triglycerides, mmol/l	1,90 ± 0,20	1,78 ± 0,07	1,81 ± 0,17
Direct bilirubin, μmol/l	3,50 ± 0,28	2,85 ± 0,06*	2,99 ± 0,11
AST, u/l	290,50 ± 13,16	308,60 ± 12,45	288,23 ± 8,86
ALT, u/l	9,75 ± 0,60	7,52 ± 0,45**	9,27 ± 0,24
Creatinine, μmol/l	50,84 ± 1,23	49,74 ± 0,72	50,21 ± 1,20
Calcium, mmol/l	1,582 ± 0,032	1,496 ± 0,022*	1,610 ± 0,009
Phosphorus, mmol/l	1,322 ± 0,066	1,242 ± 0,067	1,165 ± 0,025*

The level of transamylases in the blood of all groups, despite the decrease in the level of ALT by 12.87% ($P \geq 0.999$) in the 1st experimental group, was within the physiological norm, as was creatinine, which confirms the absence of a negative effect of the modified compound feed on the body of quails in the experimental groups.

CONCLUSIONS

1. Compared with native grain in the control group, with bioactivation in the wheat grain of the diet of the 1st and 2nd experimental groups, sugar increases from 4.99 to 8.17 and 7.20%, with a decrease in starch from 76.23 to 72.49 and 71.95%, an increase in fat levels from 1.35 to 1.51 and 1.74%. The best indicators were noted in the 2nd experimental group.

2. The inclusion of bioactivated grain in the main diet of quails in the 1st experimental group contributes to an increase in viability by 4.3%, in the 2nd experimental group – by 7.1% compared to the control group; live weight for 60 days of the experiment by 6.0 and 7.2%, respectively ($P \geq 0.99$), average daily gain – by 6.5 and 7.4% ($P \geq 0.99$), a decrease in feed costs from 7.0 to 8.5%.

3. A positive effect of bioactivated grain with sodium selenite (Group 2) on meat quality was established: a decrease in muscle tissue moisture by 1.33% ($P \geq 0.99$), an increase in dry matter by 1.31% ($P \geq 0.99$) compared to the control. In both experimental groups, bioactivation contributes to an increase in the content of essential and non-essential amino acids, including lysine – from 0.73 ($P \geq 0.99$) to 0.79% ($P \geq 0.99$), in the mineral part of muscle tissue an increase in crude ash was noted in the 1st experimental group by 0.16 ($P \geq 0.999$), in the 2nd – by 0.10% ($P \geq 0.99$), as well as calcium and phosphorus.

4. The economic efficiency of growing quails in the 1st and 2nd experimental groups was respectively 46.6 and 71.8% higher compared to the control.

5. The positive effect of bioactivated grain in the composition of compound feed on reproduction indicators has been established:

– laying hens of the 1st and 2nd experimental groups began laying eggs 3 days earlier than in the control group;

– more eggs were obtained during the record period from quail laying hens of the 2nd experimental group – 72.48 pieces, which exceeds the indicators of the control group by 8.7% and the 1st experimental group by 7.8%;

– in terms of egg mass, the laying hens of the 2nd experimental group exceeded the control by 8.2%;

– during incubation of eggs from quail laying hens of the 2nd experimental group, incubation waste was 3.37% less compared to both the control and the 1st experimental groups.

6. The use of bioactivated wheat grain in the diets of laying hens in the experimental groups had a positive effect on the quality of eggs. The thickness of the shell in the experimental groups was 10 μm higher ($P \geq 0.95$) compared to the control group.

7. The economic effect from the production of commercial eggs was higher in the 2nd experimental group by 13.96% compared to the control group.

8. A positive effect of the inclusion of bioactivated wheat grain in the amount of 20.00 wt.% of the wheat level in compound feed for quails aged 5 months on homeostasis was established:

– in the samples of the 2nd experimental group, an increase in protein levels by 24.5% ($P> 0.999$), globulins by 41.9% ($P \geq 0.999$) with a tendency to increase albumins by 4.7% ($P=0.905$) compared to the control was noted;

– the decrease in uric acid content in the 1st and 2nd experimental groups was respectively 27.3 ($P \geq 0.999$) and 15.8% ($P \geq 0.95$), which indicates good metabolism of nitrogen-containing compounds in the body of quails of these groups.

The replacement of 20.0% of native wheat grain with bioactivated grain with sodium selenite compared to bioactivated grain with water was more effective in terms of its impact on the indicators of viability, productivity of quails, the quality of their products, feed costs and economic indicators in the studied age periods.

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Распространение микоплазмоза крупного рогатого скота на территории сельскохозяйственных предприятий Вологодской области

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Исследование проведено на базе Вологодского филиала Федерального научного центра «Всероссийский научно-исследовательский институт экспериментальной ветеринарии им. К.И. Скрябина и Я.Р. Коваленко Российской академии наук» в 2022–2024 гг. С целью изучения эпизоотической обстановки по микоплазмозу крупного рогатого скота обследованы сельскохозяйственные предприятия Вологодской области. Проведены бактериологические и молекулярно-генетические исследования образцов крови, назальной, цервикальной, конъюнктивальной и препуциальной слизи от телят, спермы от быков, молока от коров, а также патолого-анатомического материала. При молекулярно-генетическом исследовании (метод ПЦР) средний показатель обнаружения геномов возбудителей микоплазмоза за неполных три года (по май 2024 г.) составил 66,3% с колебаниями по годам от 60,0 до 70,2%. Суммарный показатель по годам и видам биоматериала варьировал от 0 (сперма, кровь) до 100,0% (препуциальная слизь) с высокой долей обнаружения микроорганизмов в образцах конъюнктивальной, назальной слизи и патматериала (77,8; 72,0 и 75,0% соответственно). Бактериологическим методом исследованы 393 пробы. При этом число положительных образцов распределилось следующим образом: 48,2% – конъюнктивальная слизь, 46,8 – патматериал, 33,3 – препуциальная слизь, 26,2 – назальная слизь, 2,5% – цервикальная слизь. Из 21 положительной пробы, исследованной методом ПЦР, рост колоний микоплазм на твердой питательной среде выявлен только в 17 образцах (81,0%), из них типичные колонии в виде «яичницы-глазуньи» обнаружены в 10 образцах (47,6%), нетипичные – в 7 (33,3%). Типичными формами оказались культуры *Mycoplasma bovis*, нетипичными – *Mycoplasma dispar*. В одном из обследованных хозяйств во всех пробах патологического материала выявлен геном *M. dispar*, в другом хозяйстве во всех пробах назальной слизи идентифицирована ДНК *M. bovis* и *M. dispar*.

Ключевые слова: микоплазмоз, крупный рогатый скот, *Mycoplasma bovis*, *Mycoplasma dispar*

Spread of mycoplasmosis in cattle on the territory of agricultural enterprises of the Vologda region

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The study was conducted on the basis of the Vologda branch of the Federal Scientific Center "All-Russian Institute of Experimental Veterinary Medicine named after K.I. Skryabin and Ya.R. Kovalenko of the Russian Academy of Sciences" in 2022–2024. Agricultural enterprises of the Vologda re-

gion were surveyed in order to study the epizootic situation on bovine mycoplasmosis. Bacteriological and molecular genetic studies of blood, nasal, cervical, conjunctival and preputial mucus samples from calves, semen from bulls, milk from cows, as well as pathological and anatomical material were carried out. In molecular genetic testing (PCR method), the average detection rate of mycoplasmosis pathogen genomes for the partial three years (through May 2024) was 66.3% with year-to-year variation from 60.0 to 70.2%. The cumulative rate by year and biomaterial type ranged from 0 (semen, blood) to 100.0% (preputial mucus) with a high proportion of microorganisms detected in conjunctival, nasal mucus and pat material samples (77.8; 72.0 and 75.0%, respectively). 393 samples were analyzed by the bacteriological method. The number of positive samples was distributed as follows: 48.2% – conjunctival mucus, 46.8 – pathological material, 33.3 – preputial mucus, 26.2 – nasal mucus, 2.5% – cervical mucus. Out of 21 positive samples examined by PCR, growth of mycoplasma colonies on solid nutrient medium was detected only in 17 samples (81.0%), of which typical colonies in the form of "fried egg" were found in 10 samples (47.6%), atypical colonies were detected in 7 samples (33.3%). *Mycoplasma bovis* cultures were typical forms, *Mycoplasma dispar* cultures were atypical. In one of the surveyed farms, the *M. dispar* genome was detected in all the samples of pathological material; in another farm, DNA of *M. bovis* and *M. dispar* was identified in all the samples of nasal mucus.

Keywords: mycoplasmosis, cattle, *Mycoplasma bovis*, *Mycoplasma dispar*

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INTRODUCTION

Mycoplasmosis of cattle causes significant economic damage to livestock farms, being one of the reasons for the low yield of calves and the causative agent of a number of diseases (keratoconjunctivitis, rhinitis, pneumonia, arthritis, mastitis, etc.)[1] [1]. In addition, mycoplasmosis has a negative impact on the process of animal reproduction - it causes infertility, early abortions, etc. The methods of specific treatment and prevention of the disease in question require further optimization, therefore the study of mycoplasmosis in specific conditions of place and time is very relevant today [2, 3].

Mycoplasmas are the smallest prokaryotic microorganisms that do not have a cell wall and are resistant to antibiotics, the main mechanism of action of which is to suppress the process of synthesis of the bacterial wall. The predominant route of transmission of mycoplasmosis is aerogenic. In natural conditions, transmission often occurs through alimentary, sexual and transplacental routes[2] [4].

[1] *Abed A.M.* Epidemiological study of mycoplasma mastitis in cattle (transmission and control) // Innovative approaches in modern science: collection of articles based on the materials of the LXXV International scientific and practical conf. Moscow, 2020, vol. 15 (75), pp. 7–13.

[2] *Alekseeva K.N., Kozlov Yu.V.* Experience of using tulatromycin in the prevention of mycoplasmosis in cattle // Scientific support for the agro-industrial complex: collection of articles based on the materials of the 78th scientific and practical. students' conference on the results of research for 2022. Krasnodar, 2023, Part 1, pp. 290–293.

The most common pathogenic and clinically significant types of mycoplasma isolated from young and adult cattle currently include *Mycoplasma bovis*, *M. dispar* and *M. bovigenitalium* [5]. The most pathogenic among the listed species is *M. bovis* [6]. High contagiousness of some types of mycoplasma, complexity of treating mycoplasmosis and significant economic costs of culling affected livestock determine the relevance of timely and accurate diagnostics for disease control and prevention[3] [7, 8].

Diagnosis of mycoplasmosis is based on the analysis of epizootological, clinical and pathological-anatomical data, as well as on the results of laboratory studies, including cultivation, molecular genetic studies, electron microscopy, etc.[4, 5] [9]. Cultivation of mycoplasmas on selective nutrient media is a classic and least expensive method, but the most labor-intensive task, since specialized media and microaerophilic conditions are required for the growth of mycoplasmas [10–13]. Identification of mycoplasma isolates to the species level is also critical, as *M. bovis*, *M. dispar* and *M. bovigenitalium* are considered primary pathogens, while other species are considered part of the resident microbiome that does not play a significant role in disease development[6].

Despite the large number of nutrient media with different compositions recommended for the isolation and cultivation of cattle mycoplasmas, to date no standard, universal media produced by the biological industry have been created with the help of which it would be possible to carry out the indication and identification of microorganisms quickly enough [14].

Effective diagnostics allows for the prompt introduction of early measures to control the herd for mycoplasmosis. In this regard, at the present stage, the most important task of researchers is the initial detection of mycoplasma strains that have a pathogenic effect on cattle, followed by their identification.

The purpose of the research is to study the epizootic situation for mycoplasmosis in cattle on the territory of agricultural enterprises in the Vologda region with the determination of the species affiliation of isolated crops.

MATERIAL AND METHODS

The study was conducted by specialists from the Vologda Branch of the Federal Scientific Center "All-Russian Institute of Experimental Veterinary Medicine named after K.I. Skryabin and Ya.R. Kovalenko of the Russian Academy of Sciences" in 2022–2024. The object of the study is the samples of biomaterial from bulls, cows and calves provided by agricultural enterprises of the Vologda Region.

The molecular genetic method (RT-PCR) was used to study the blood samples (n = 5), nasal (n = 50), cervical (n = 18), conjunctival (n = 9), preputial (n = 1) mucus from the calves, semen from the bulls (n = 1), pathological material (lung tissue, mediastinal lymph nodes from calves, aborted fetuses) (n = 8) from seven agricultural enterprises.

The material for bacteriological examination was nasal (n = 191), cervical (n = 40), conjunctival (n = 29) and preputial mucus (n = 9), bull semen (n = 15), cow milk (n = 77), pathological material (lung tissue, mediastinal lymph nodes, aborted fetuses) (n = 32) from 11 agricultural enterprises.

The studies were conducted in accordance with the “Methodological recommendations for the isolation, cultivation and identification of mycoplasmas, acholeplasmas and ureaplasmas”[7].

[3]*Zubova T.V., Smolovskaya O.V., Semechkova A.V.* Cultural identification of mycoplasmas // Modern trends in agricultural production in the global economy: Proc. XX Int. scientific-practical. conf. Kemerovo, 2021, pp. 417–422.

[4]*Makavchik S.A., Vaganova A.N., Sukhinin A.A., Verbov V.N., Sboychakov V.B., Borisenko S.V., Roka V.V.* Laboratory methods for diagnosing infections caused by *Mycoplasma bovis, Mycoplasma bovigenitalium* and *Ureaplasma diversum:* method. recommendations. St. Petersburg, 2023, 50 p.

[5]*Doronin M.I., Lozovoy D.A., Mikhalishin D.V., Starikov V.A., Guseva M.N., Borisov A.V., Volkov M.S.* The use of Novostimin for decontamination of mammalian cell lines from mycoplasmas // Proceedings of the Federal Center for Animal Health. Vladimir, 2020, vol. 17, pp. 225–239.

[6]*Shastin P.N. Mycoplasma bovis* – pathogen, diagnostics and prevention // Actual problems of science and technology. Innovation: collection of scientific articles based on the materials of the X International scientific and practical conference. Ufa, 2023, pp. 7–12.

[7]Guidelines for the isolation, cultivation and identification of mycoplasmas, acholeplasmas, ureaplasmas. Moscow, 1982, 48 p.

Molecular genetic testing was carried out at the Vologda Regional Veterinary Laboratory and the Federal Center for Animal Health (VNIIZZH, branch in Vladimir) using the primers for detecting mycoplasma DNA (PCR-mycoplasmosis-factor reagent kit).

During bacteriological examination, the material was seeded on liquid and solid nutrient media (PPLO, AGV) with the addition of glucose, horse blood serum, and yeast extract. To suppress the growth of foreign microflora, antibacterial drugs (penicillin, vancomycin) and fungicides (amphotericin, fluconazole) were added to the nutrient media.

The obtained data were statistically processed using the Microsoft Office 2010 software package.

RESULTS AND DISCUSSION

According to the information of the Veterinary Department of the Vologda Region, mycoplasmosis of cattle in livestock farms of the region up to and including 2021 was not registered. According to our data, individual herds of cattle in agricultural enterprises of the region are affected by mycoplasmosis, which is confirmed by the results of bacteriological and molecular genetic studies.

In 2022–2024, 92 samples of biomaterial from one bull and calves of different ages with mycoplasmosis symptoms (nasal and conjunctival discharge, cough, etc.) were tested for mycoplasmosis using the PCR method in the Vologda Regional Veterinary Laboratory. The results are presented in Table 1.

It was found that the average detection rate of mycoplasma pathogen genomes for less than three years (up to May 2024) was 66.3%, with annual fluctuations from 60.0 to 70.2%. The total rate by year and type of biomaterial varied from 0 (sperm, blood) to 100.0% (preputial mucus) with a high percentage of confirmation in samples of conjunctival, nasal mucus and pathological material (77.8; 72.0 and 75.0%, respectively). In the context of farms, the proportion of positive results for all types of biomaterial varied from 0 (two farms) to 100.0% (two farms).

The obtained data indicate a fairly high percentage of mycoplasmosis detection by the PCR method, especially when examining pathological material, nasal and conjunctival mucus (up to 100.0% in 2022).

In 2022–2024, 393 samples were examined using the bacteriological method. The results of the study are presented in Table 2.

According to the data obtained, the percentage of detection of the colonies in the form of "fried egg" typical for mycoplasmosis (see Fig. 1) on average over the study period was 6.6% with fluctuations by year from 1.8% in 2024 to 12.3% in 2023, and by farms – from 0 to 75.0%. In addition to typical colony shapes, forms in the form of "sunflowers", "volcanoes with craters", etc. were detected (see Fig. 2).

The distribution of positive samples by type of biomaterial for the entire study period was as follows: 26.2% (colonies in the form of "fried egg" – 13.1%) – nasal mucus, 46.8 (3.1) – pathological material, 48.2 – conjunctival mucus, 33.3 – preputial mucus, 2.5% – cervical mucus. Moreover, when studying nasal mucus samples, the greatest number of cases of detection of mycoplasma colonies with a characteristic growth in the form of "fried egg" was noted. Mycoplasma colonies could not be isolated from milk and sperm.

When comparing the results obtained by both methods, the following was established. Of the 21 positive samples confirmed by the PCR method, colony growth on a solid nutrient medium was recorded only in 17 samples (81.0%), including colonies in the form of "fried egg" – in 10 samples (47.6%), atypical forms – in 7 samples (33.3%).

Later, the species affiliation of typical and atypical forms of colonies was established in the reference laboratory of cattle diseases of the All-Russian Research Institute of Animal Health. Typical forms were *M. bovis* cultures, atypical – *M. dispar*. In one of the examined farms, the *M. dispar* genome was detected in all samples of pathological material, in another farm, *M. bovis* and *M. dispar* were found in all samples of nasal mucus.

The identified colonies of *M. bovis* and *M. dispar* were examined for biochemical prop-

Табл. 1. Результаты исследования биоматериала методом ПЦР

Table 1. Results of biomaterial testing by PCR

Number of farms/ of which surveyed for the first time	Number of samples															
	Nasal mucus		Cervical mucus		Conjunctival mucus		Preputial mucus		Sperm		Blood		Pathological material		For all types of biomaterials	
	Total	Positive, pcs./%	Total	Positive, pcs./%	Total	Positive, pcs./%	Total	Positive, pcs./%	Total	Positive, pcs./%	Total	Positive, pcs./%	Total	Positive, pcs./%	Total	Positive, pcs./%
2022																
3/3	18	17/94,4	18	11/61,1	2	2/100,0	1	1/100,0	1	0	5	0	2	2/100,0	47	33/70,2
2023																
4/2	15	8/53,3	0	0	7	5/71,4	0	0	0	0	0	0	3	3/100,0	25	16/64,0
2024 (from January till May)																
4/2	17	11/64,7	0	0	0	0	0	0	0	0	0	0	3	1/33,3	20	12/60,0
2022–2024																
11*/7	50	36/72,0	18	11/61,1	9	7/77,8	1	1/100,0	1	0	5	0	8	6/75,0	92	61/66,3

*During the study, some farms were re-examined.

Табл. 2. Результаты бактериологического исследования биоматериала

Table 2. Results of bacteriological examination of the biomaterial

Number of farms/ of which surveyed for the first time	Number of samples															
	Nasal mucus		Cervical mucus		Milk		Conjunctival mucus		Preputial mucus		Pathological material		Sperm		For all types of biomaterials	
	Total	Positive, pcs./%	Total	Positive, pcs./%	Total	Positive, pcs./%	Total	Positive, pcs./%	Total	Positive, pcs./%	Total	Positive, pcs./%	Total	Positive, pcs./%	Total	Positive, pcs./%
2022																
4/4	54	4/7,4**	35	0	30	0	6	0	1	0	1	1/100,0**	0	0	127	5/3,9**
2023																
10/3	67	32/47,8 (19/28,3**)	2	1/50,0	44	0	16	8/50,0	5	3/60,0	14	7/50,0 (1/7,1**)	15	0	163	51/31,3 (20/12,3**)
2024 (from January till May)																
7/4	70	14/20,0 (2/2,8**)	3	0	3	0	7	6/85,7	3	0	17	7/41,2	0	0	103	27/26,2 (2/1,8**)
2022–2024																
21*/11	191	50/26,2 (25/13,1**)	40	1/2,5	77	0	29	14/48,2	9	3/33,3	32	15/46,8 (1/3,1**)	15	0	393	83/21,1 (26/6,6**)

*During the study, some farms were re-examined.

** Mycoplasma colonies with characteristic "fried egg" growth pattern.

erties using a number of tests: inoculation on a medium with Tween 80, arginine hydrolysis, and blood serum liquefaction. It was found that the isolated mycoplasma species do not cause arginine hydrolysis, do not liquefy blood serum, and do not form a light ring on a medium with Tween 80, which allowed them to be classified as pathogenic. The identified *M. bovis* culture, unlike *M. dispar*, was characterized by the presence of phosphatase activity, which is applicable as a criterion for differentiating the indicated microorganism species.

Since *M. bovis* and *M. dispar* cause many diseases in cattle [15], improving their diagnostic and identification methods is very important today. When using the PCR method, detection of mycoplasma genetic material in a sample is not direct evidence of the presence of a living pathogen capable of causing disease, which casts doubt on the correctness of the diagnosis [2]. In this regard, the indication and identification of mycoplasmas affecting adult individuals and calves is carried out primarily using bacteriological methods with the use of special nutrient media. Mycoplasmas can be differentiated by the bacteriological method not only by the forms of colonies, but also by their biochemical properties. Thus, according to M. Abed Alhussen et al. [15], *M. dispar* does not produce colonies typical of mycoplasmas, especially in early passages, while *M. bovis* forms typical forms in the form of "fried egg".

CONCLUSION

The obtained results indicate the spread of mycoplasmosis of cattle in the farms of the Vologda region. Identification of the genomes of *M. bovis* and *M. dispar* in the intravital and

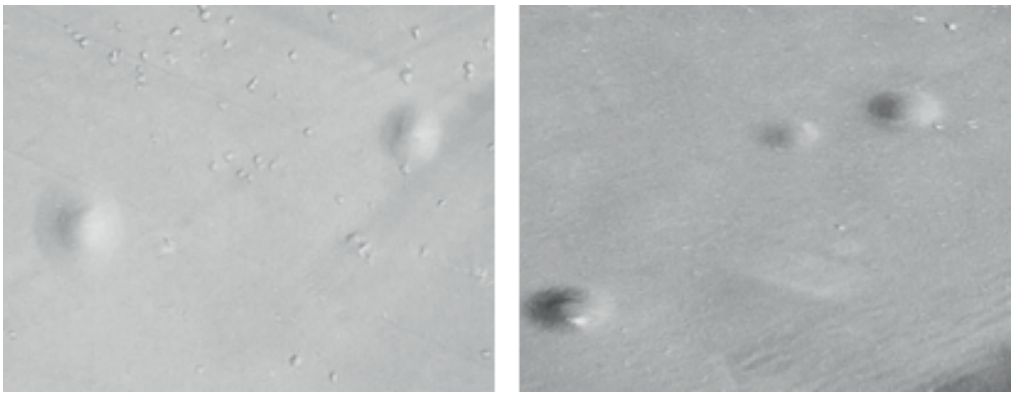

Рис. 1. Типичная форма колоний микоплазм в виде «яичницы-глазуньи»
Fig. 1. Typical shape of mycoplasma colonies in a "fried-egg" form

а *б*

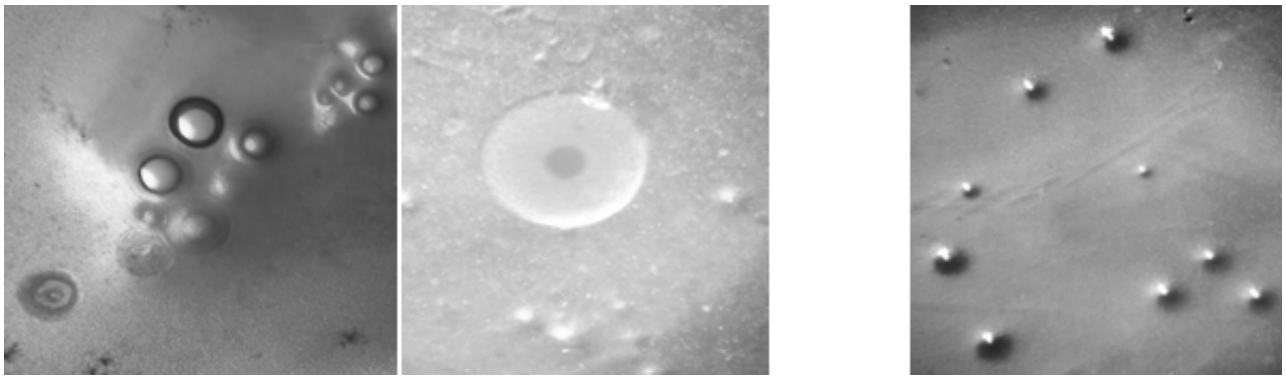

Рис. 2. Нетипичные формы колоний микоплазм в виде «подсолнухов» (*а*) и «вулканов с кратерами» (*б*)
Fig. 2. Atypical forms of mycoplasma colonies in the form of "sunflowers" (*а*) and "volcanoes with craters" (*б*)

postmortem material of sick animals indicates the need to take a set of measures to prevent the further spread of pathogenic mycoplasma species and prevent this infection on the territory of agricultural enterprises.

The results of PCR diagnostics make it possible to establish the cause of the disease at the gene level. However, one should not underestimate the importance of bacteriological methods for studying mycoplasmosis, which allow one to determine the presence of a living cell in the studied biomaterial and its role in the occurrence of pathology in animals.

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