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Preface

The collection presents the reports of participants of the Global Food Forum organized by Moscow State University of Food Production (MSUFP) jointly with the Council for Science and Continuing Education of the Eurasian Peoples' Assembly, with the support of the Federation Council Committee on Agriculture and Food Policy and Environmental Management of the Federal Assembly and the assistance of Moscow Office of the Food and Agriculture Organization.

The Global Food Forum 2021 became a venue for wide-ranging discussion of plans and actions realised in the Russian Federation and a number of foreign organisations to achieve the Sustainable Development Goals. A number of proposals were made towards coordination of inter-sectoral actions along the entire chain of food systems (production, transportation, storage, distribution and consumption), drawing special attention to the problems coupled with Sustainable Development Goals in scientific research, their expansion and allocation of necessary resources for these purposes, training of required personnel, including highly qualified staff.

The Forum was attended by representatives of 28 universities and research institutes from such countries as: Russia, Belarus, Kyrgyzstan, Iran, Uzbekistan, Germany, Azerbaijan, Turkey, Kazakhstan, China, Tajikistan, Bulgaria and the UAE.

The global attention to the Forum is accounted for by the importance of uniting world community efforts for identification and prevention of internal and external threats to food security, for development of common constructive decisions on improvement of food systems, on achieving progress, through the food resource, in respect of all 17 UN Sustainable Development Goals with the view of sustainable reproduction of healthy and full-value life.

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Environmental and Economic Efficiency of Water Subsidies for Irrigation in Kazakhstan

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Abstract. Most of the country's land is located in a harsh continental arid type of climate. Many agricultural crops are not able to develop normally with a lack of precipitation and low soil moisture. In this case, the ecological effect of irrigation comes to the fore. The expansion of the area of irrigated land in Kazakhstan requires the revision of the water subsidy mechanism. The reason for it is insufficient ecological and economic efficiency in the conditions of primitive irrigation technologies. They put a lot of pressure on the environment. The purpose of the study is to propose and justify recommendations on the increase of the economic efficiency of water subsidies for irrigated agriculture in Kazakhstan. Methods: calculation and statistical, comparative, time series analysis. The paper is based on the cost approach. It is applied for the analysis of the environmental and economic efficiency of subsidies aimed at the increase of the economic accessibility of water. The results and novelty consist in the assessment of the environmental and economic efficiency of water subsidies using adjusted indices. The lack of focus on the used irrigation technology reduces the ecological and economic efficiency of water subsidies in the long term. The dynamics of the Water Resource Exploitation Index coincides with the trend of subsidizing water for irrigation. This relationship indicates that subsidies only increase the pressure on water resources. Thus, there are no clear incentives for the introduction of advanced technologies in Kazakhstan. They could increase the competitiveness of irrigated agriculture, though.

Introduction

Kazakhstan experiences shortage of water resources. That is why the problem of their rational use is crucial. It concerns many foreign countries as well. That is confirmed by numerous publications in both domestic and foreign periodicals.

Scientists' views on the environmental and economic efficiency of water subsidies differ. The keynote of many scientific works is the necessity of transition from extensive to intensive type of consumption in order to conserve water in Kazakhstan and other countries of Central Asia [1-2].

M. Gallaher et al. [3], having studied the experience of the United Arab Emirates, claim that subsidies on resources (in particular, on electricity and water) stimulate their excess consumption. Subsequently, it harms the environment. According to B.G. Delworth and R.G. Huffaker [4], water pricing for irrigated agriculture does not require a government role. On the contrary, they recommend the market mechanism for determining the price of water.

Both government support and the control of environmental pollution caused by irrigated agriculture can be included in expenditures. Financial and economic performance indicators are not always reflected from an environmental perspective. The importance of the principle of safety for the environment is often neglected. Based on the opinion of A.I. Borodin, N.N. Kiseleva and N.N. Shash [5], we can conclude that the ecological effect is considered as the total amount of resources required to eliminate the consequences of water resources depletion due to irrigation. OECD defines water use efficiency as the ratio of value added to water used [6].

T.N. Slepneva [7] provides definitions of technological, economic, social and environmental efficiency in agriculture. She insists that it is also important to analyze the cost of eliminating or preventing the negative effects of agricultural production on the environment.

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T.I. Espolov and M. Musaeva [1] note that subsidizing water charges does not encourage Kazakhstani farmers to use water resources rationally. This statement is taken as the hypothesis of our research.

The purpose of the study is to propose and substantiate recommendations on increasing the economic efficiency of water subsidies for irrigated agriculture in Kazakhstan.

The objectives of the research: 1) to analyze the ecological and economic efficiency of the current system of subsidizing water for irrigated agriculture in Kazakhstan; 2) to assess the economic accessibility of water for irrigated agriculture in the republic.

The novelty of the study consists in the assessment of environmental and economic efficiency of water subsidies using adjusted indices. In addition, recommendations for improvement of the environmental and economic efficiency of the existing subsidy mechanism are provided.

Materials and Methods

The calculations in this work are based on a cost approach. It is convenient for studying water tariffs and compensations to farmers for its delivery. The advantages and disadvantages of this approach are described in the work [8]. The environmental pressure due to irrigated agriculture is determined by using special indicators. Bureau for National Statistics [9] does not distinguish irrigation from water abstraction. In connection with this omission, we have adjusted the existing indicators. Water Resources Exploitation Index (*WRE*) is calculated as the quotient of dividing the volume of water used for irrigation (*I*) by the volume of the country's freshwater resources (*F*):

$$WRE = \frac{I}{F} \cdot 100\%, \tag{1}$$

Pressure on Water Resources Index (due to irrigation) (*PWR*) is calculated as the ratio of the volume of water used for irrigation (*I*) to the volume of freshwater resources (*F*) minus the ecological demand for water (E = 36.31 km^3, according to AQUASTAT [10]):

$$PWR = \frac{I}{F - E} \cdot 100\%, \tag{2}$$

The data on the areas of irrigated land required for the calculations were extracted from Bureau of National statistics [9]. Data on water resources were taken from the statistical compilations "Environmental Protection in the Republic of Kazakhstan". The Committee on Water Resources of the Ministry of Ecology, Geology and Natural Resources compiles a series of such data. Water demand for irrigation needs is analyzed by the calculation method shown in [6].

The ecological and economic efficiency of water subsidies was assessed by the calculation and statistical method, which will be demonstrated in Table 1.

Results

Subsidizing the cost of water delivery services in Kazakhstan at current prices was increasing during 2011–2020. However, in terms of constant 2008 prices, the amount of subsidies was unstable. The Water Resources Exploitation Index for irrigation and the amount of subsidies changed in similar directions. In 2019, it reached 9.6%, which reflected the increased environmental pressure. The Pressure on Water Resources Index (due to irrigation) in 2009-2019 slightly fluctuated and was in the range of 18-21% (Table 1).

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TABLE 1. Subsidizing the cost of water delivery services to agricultural producers and the pressure on water resources for irrigation in Kazakhstan in 2008-2020

Indicator	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020
The amount of subsidies paid in 2008 prices, US$ millions	6.4	8.6	8.8	6.8	7.0	6.7	7.6	5.4	2.1	1.7	2.1	1.8	1.8
Fresh water volume for irrigation needs, billion m³	8.16	8.89	9.05	9.07	8.84	9.49	9.49	9.83	9.02	9.51	9.49	10.30	n/a
Renewable fresh water resources, billion m³	89.7	100.0	180.8	99.8	78.4	131.3	108.1	115.6	160.0	122.1	110.7	107.6	n/a
Irrigation Water Exploitation Index, %	9.1	8.9	5.0	9.1	11.3	7.2	8.8	8.5	5.6	7.8	8.6	9.6	n/a
Pressure on Water Resources Index (due to irrigation), %	16.8	18.3	18.6	18.6	18.2	19.5	19.5	20.2	18.5	19.5	19.5	21.2	n/a

Source: compiled by the authors based on the materials [9, 11, 12].

Under conditions of rainfed agriculture, farmers can earn US$100-125 per 1 ha. Then, on irrigated land with modern irrigation technologies, farmers can get US$1.2-1.5 thousands per 1 hectare for individual crops. That is, the profitability per 1 hectare of irrigated land rises quite significantly.

From the authors' point of view, in order to increase the efficiency of the used irrigated lands, it is necessary to focus on the following measures:

1. Transition from water-intensive agricultural crops (rice, cotton) to less water-intensive (technical, fruit and berry), the introduction of scientifically based crop rotations;
2. Restoration, reconstruction and comprehensive modernization of hydro-melioration systems;
3. Subsidizing agricultural producers aimed at implementation of annual reclamation activities to clean irrigation and collector-drainage networks, deep soil loosening, capital planning of fields;
4. Improvement of the agricultural subsidy system for the use of irrigation water and introduction of drip irrigation technology. In 2019, it covered only 6.1% of the country's irrigated land [13];

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5. Raising the standards of subsidies for irrigated agriculture while subsidizing the production of priority crops in connection with uneven seasonal distribution of the costs of agricultural producers;
6. Further creation of rural consumer cooperatives of water consumers, strengthening their material and technical basis by providing preferential loans and leasing for the purchase of special equipment for land reclamation work;
7. Using opportunities of rural credit partnerships in financing reclamation activities;
8. Providing personnel training, improving their qualifications and training rural producers to work with modern irrigation technology, water metering systems, as well as introducing agricultural producers to other issues of effective water use and agricultural production on irrigated lands;
9. Support for the operation and maintenance of vertical drainage networks;
10. Enhancing the role of agricultural science in conducting comprehensive research to improve the efficiency of irrigated agriculture and agricultural water use.

The current tariff policy in the field of water resources discourages the introduction of environmentally friendly technologies and investments in the field of water resources management. To solve this problem, it is proposed to start a revision and differentiated establishment of an increased tariff for water for agricultural producers who consume water excessively. For manufacturers introducing drip irrigation technologies, it is recommended to raise water subsidy standards.

Discussion

The study shows the importance of the introduction of advanced technologies for fields irrigation in the republic. The conclusions of the article do not contradict the OECD report [14], which proclaims the necessity to increase the return on water use in agriculture in Kazakhstan. V. Dhawan [15], OECD [14] emphasize the importance of infrastructural upgrade in land irrigation. In contrast to our study, Le Blanc [16], L.A. Andres et al. [17] focus on monopoly prevention in the water supply market. At the same time, our article does not identify the harm from the natural monopoly represented by the RSE “Kazvodkhoz”. We see no threat from such a monopoly, especially since it has not set high water tariffs. L.A. Andres et al. [17] indicate that the state should cease its involvement in water supply services in the future.

This measure is necessary in order to avoid supplanting private initiative in the market. This recommendation was not reflected in our work. C. Chatterjee et al. [8], H. Salunkhe [18] use the example of India to explain the harm from subsidizing irrigation water. It causes an increase in water use, which leads to groundwater depletion. We also lay emphasis on the environmental impact of the policy, therefore we offer water saving (in particular, drip irrigation). I.P. Aydarov and E.I. Pankova [2] highlight drip irrigation and sprinkling as the most rational ways of watering plants (in providing them with moisture and saving water). For the most part, our findings are compatible with those of other researchers. In the prospect, we will continue to explore the above aspects.

Conclusion

To solve the problem and to create conditions for increasing irrigated areas, it is necessary to develop a unified pricing policy for irrigation water and provide an investment component in the tariff. These measures must be taken at the level of the Committee for the Regulation of Natural Monopolies and Protection of Competition of the Ministry of National Economy of the Republic of Kazakhstan. In addition, it is essential to develop organizational and economic mechanism for reconstruction and utilization of fixed assets of this sub-industry. The annual decrease in water resources in Kazakhstan, which is attributed to outdated water supply systems, leads to a slowdown in the development of crop production. It is necessary to implement a comprehensive water-saving programme with the use of modern water supply technologies. It is recommended that subsidies be redirected towards the purchase of drip irrigation equipment.

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Ecology-Oriented Agriculture: Expert Assessments

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Abstract. Public demand for systematic socio-economic sustainability predetermines the relevance of environmental research. The article aims to analyze the perception of ecological orientation in agricultural production, trade, and consumption. The discussion is based on the authors' qualitative data gathered in 2019-2020 in Tyumen Oblast, Russia. Empirical base includes 110 in-depth interviews with rural residents in agriculture-related occupations (representatives of business, authorities, production, trade, community). Results show a significant gap between the official environmental agenda and its public depiction, as two opposite standpoints coexist in the discourse of regional actors in agribusiness. Environmental topics turn out to be poorly supported during dialogue, as not every expert agrees to discuss them outside the frame of an idle talk. Expert perceptions reveal factors that restrict the ecological orientation of agriculture: economic, mental, cultural, managerial. Leaving small private farms out of brackets, the perception of pro-ecological agriculture is associated more with negative assessments and resistance than the desire to employ and develop the best practices.

INTRODUCTION

Agricultural intensification causes risks for the environment and human health. The demand for sustainability and development of society is increasing, that is why research in the field of ecological orientation of agriculture is becoming substantially relevant [1, 2].

In turn, sustainability and development of agriculture are linked to the concept of food system, which combines environment, people, resources, processes, infrastructure and institutions, when addressing socio-economic and environmental factors of production, storage, processing, preparation, distribution, marketing, trade and consumption of food [3, 4]. In this context, consumer behavior depends on 1) places of food sale (outlets); 2) infrastructure of access to these places; 3) personal determinants of choice (income, education, values, skills); 4) political, social and cultural norms. The food system is formed under the influence of the physical, economic, political and socio-cultural context in which consumers and producers interact within institutional rules [5, 6].

Agriculture, ecology and society can no longer be considered separately, since the complex processes of their interrelation form an agroecosystem. Agroecosystems depend on power decisions at different levels, which range from individual farmers and entrepreneurs on a local scale to government bodies on a national scale. The choice of approach to agribusiness (traditional or ecologically oriented) affects natural environmental processes on different levels: from an individual farm, to a region and even a country [7]. For example, the selection of new agricultural crops using intensive breeding methods has led to the significant dependance of cultivated plants on nutrients coming from outside, compared with wild species [8]. An increase in biomass production on a plot of land leads to the abiotic and biotic modification and subsequently affects the nutrients in the soil; the use of pesticides can harm potentially beneficial microflora; some tillage methods can lead to a decrease in the number of soil microorganisms 9, 10].

In general, there are five groups of factors that hinder the ecological transformation of agriculture: 1) government factors; 2) economic factors; 3) knowledge factors; 4) social and cultural factors; 5) resource factors [11]. In this regard, it is especially important to study the interaction of environment, society and production.

Because, on the one hand, ecological agricultural production can be safer for the environment and produce more nutritious and healthy products [12, 13], on the other hand, ecological productions may have yield variability over time, since they cannot rely entirely on their internal resources [14]. Studies show that organic producers have a narrower range of control methods and may be more affected by changes in natural conditions. Variability in yields means instability of profits [15].

Despite these shortcomings, the improvement of agriculture is carried out in accordance with the latest achievements in the theory and practice of agroecosystems. Therefore, it is especially important to understand the perception of environmental agenda by people who directly influence the implementation of state, regional and municipal policies and programs and take responsibility for the development of agriculture at the end point. It is necessary to find out how experts relate to the existing processes in agriculture, what their assessments of problems and prospects are, whether the experts are ready to contribute to the evolution of best practices that comply with the recommendations of international organizations and address local specifics. Thus, the article **aims** to analyze the perception of ecological orientation in agricultural production, trade, and consumption.

This article illuminates ecological problems and ecological orientation of agricultural production through expert assessments. The authors use the interview data to analyze understandings and perceptions of representatives of government, business, science, and community. The **study hypothesizes** that at the declarative level, eco-oriented activities in agriculture are developing actively and successfully, while the opinion of the informed part of the population does not correspond to this.

Materials and Methods

Empirical base of qualitative research includes 51 expert in-depth interviews of spring-summer 2019 and 59 expert in-depth interviews of spring-summer 2020, conducted in Tyumen Oblast, Russia. All experts live in rural areas and have occupations related to production, trade and consumption of agricultural products.

The 2020 sample included: executive managers (17) and owners (9) of agricultural enterprises (category “business”); representatives of municipal authorities (11) (“power”); members of the Oblast Duma from agricultural districts (3) (“authorities”); active representatives of community, leaders of public organizations (16) (“community”); professors-researchers from universities (3) (“science”).

The 2019 sample included: (9) “power”; (23) “business”; (10) “science”; (9) executive managers of rural retail outlets (“trade”).

58% of experts are men, 83% have higher education.

The interview discussed issues of rural areas, agricultural business, food security; prospects for the production and consumption of ecological “clean” food; prospects for eco-oriented activities in agriculture. Experts were asked to talk about what actions can ensure transition to eco-oriented agriculture, whether there is a need for such transition.

Results

The results will be divided into three thematic blocks, each of them will be given a short description based on expert citations and author comments.

Environmental situation

Here, expert assessments are clearly split into two polar opinions. The first opinion notes clean air, the opportunity to gather berries and mushrooms, graze cattle without the fear of contamination. Living in the city makes people appreciate the environment (although this is not relevant for the villagers). The opinion that there is no problem of environmental pollution in the region is also supported by experts from the “science” category.

“*... cities are growing, people are still drawn to the cities”* (business)

“*Now, there are no problems with everything related to the ecologization of agriculture. We do not exceed the norm of mineral fertilizers, we do not fill plants with chemicals. Organic farming has been, is and will be*”. (science)

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The second opinion notes environmental pollution. And the speed of transition between these opinions (from absolutely positive to almost panicky) creates the basis for social conflict. Which is also confirmed by estimates of the cost of an environmentally friendly product.

"*... there is a poultry farm, and people can smell it with their noses, because this miasma is a constant nightmare"*. (community)

"*God, of course not, we can't get to that... we do not have a legislative framework for environmental entrepreneurship. I think it is unlikely that it will work out in the near future. So, we are not up to ecology, we just try to feed people"*. (business)

Ecological problems are perceived as something "imposed from the outside", "unfamiliar to this territory". Experts still believe that the main actor of eco-oriented agriculture is the government. And business is seen as a victim of "constant extortion" and "tax pressure".

Ecology-oriented activities

In general, the prevailing opinion among agribusinesses is that any of their activities is environmentally oriented.

"*We do not worsen the environment in any way"*. (business)

"*Any fertilizers are only for the benefit"*. (business)

In the assessments of experts from business and trade, waste disposal is directly related to unnecessary costs, an increase in the final cost, and is perceived as a serious burden. It is noted that only bigger businesses can afford it.

"*The main problem for agricultural producers is waste. They have a lot of this "dead" waste, they don't know where to put it"*. (authorities)

In the assessments of most experts, environmental friendliness and profitability are poorly connected. Compliance with environmental standards in business is becoming almost unbearable for most local small businesses.

"*Well, it's no secret that if everything is done according to the rules, then no money will be enough"*. (business)

"*The rules are made to break them, after all. And the burial grounds were deliberately wrongly made"*. (community).

Perception of "clean", high-quality food

Expert assessments are dominated by the idea that local products are "cleaner" and more high-quality, which is probably due to the desire to support the community and local producers in their confrontation with major players. At the same time, experts did not fail to scold and criticize retail. Chain stores are accused of refusing to take products from local businesses, do not pay attention to taste, understate the price, which creates severe competition conditions for smaller companies.

In the current conditions, food gets on the dining table through a dense filter. The need to meet quality requirements is the main factor noted by both agricultural producers and trade.

"*No, today meat and dairy producers are very demanding about this. They are checked from all sides. All the state services in the world check them"*. (business)

Experts from production pay special attention to freshness and note an increase in consumer demand for quality. Interestingly, the opinion of representatives of mass retail is completely different.

"*The buyer has become more demanding… They have the opportunity to travel to other cities, abroad. Not only the price is important to them, but also quality, packaging, and freshness"* (business)

"*...high-quality "clean" products are normally more expensive, and consumers choose a low-quality product that is sold on the shelves of our federal retail chains. This, of course, greatly violates the competitiveness… People vote with their wallets, and producers of high-quality food suffer"* (trade).

Conclusion

Our study uncovers ecological agenda in agriculture and perception of ecological problems in production and consumption of food from the point of view of the informed public and business, using original empirical data.

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Reasoning based on the study of the experience and opinions of the most knowledgeable and active representatives of rural territories may indicate that the results contain elements of scientific novelty.

We highlight the contradictions between agricultural business, rural residents and authorities and deduce that these contradictions are practically ignored by the official media, according to experts. The scientific appeals, proving the benefits of eco-oriented agriculture, do not find proper agreement in the common information field.

Experts (categories "business" and "community") living in rural areas were not willing to talk about environmental problems. As a rule, to prevent a conversation from ending, informants moved on to socially approved topics (e.g., "why is it good to live in the countryside compared to the city"), while representatives of local and regional power were much more eager to discuss these topics. In these situations, the authors had a feeling that ecological theme is imposed by authorities, media, and scientific community. However, rural residents do not see environmental problems in their life, strongly believe that their activities cannot but be eco-oriented, and resist the environmental agenda as a fashionable, but completely irrelevant topic.

Thus, there is a significant gap in the official ecological discourse and real activities in agriculture. Regional authorities, agricultural businessmen share almost two opposite discourses simultaneously. The first discourse, paying tribute to the popular and state-supported environmental topic, criticizes the ecological situation and proves the need to improve it. The second discourse insists on the necessity of the paradigm "local means clean" and "we don't have bad farms and factories, so everything is fine with the environment". The assessments of the executive managers of retail outlets stand somewhat apart, because they are faced with high requirements of international quality standards, an increase in the demands of consumers, a diversity of opinions regarding possible consumer strategies.

Environmental topics turn out to be poorly supported during dialogue, as not every expert agrees to discuss them outside the frame of an idle talk. Expert perception reveals factors that restrict the ecological orientation of agriculture: economic ("it is expensive"), mental ("everything is fine as it is"), cultural ("we are not used to doing business otherwise"), managerial ("we are ordered by the authorities to control, but not to decide"). Leaving small private farms out of brackets, the perception of pro-ecological agriculture is associated more with negative assessments and resistance than the desire to employ and develop the best practices.

Acknowledgments

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Heavy Metals and Radionuclides in Animal Feed and Products

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Abstract. The Altai Republic is an agricultural region in which livestock breeding is developed. Being one of the most ecologically clean regions of Russia, Altai has recently been subjected to a serious recreational load, which cannot but affect environmental conditions. Therefore, animal products require constant monitoring, for compliance with basic safety standards. The work objective was to assess the current ecogeochemical state of the soil-water-plants-animals system, it provides data on the accumulation of heavy metals and radionuclides in organisms of wild plants and some types of farm animals selected for analysis in different altitude zones of the Altai Mountains. The method of gamma-spectrometric analysis was used, radionuclides 228Ra, 232Th, 40K, 137Cs were determined and studied on a spectrometric unit using Progress-2000 software and hardware complex. The study of trace elements and heavy metals (Cu, Pb, Co, Mn, Fe) was carried out on QUANTUM-2 atomic absorption spectrometer by atomic absorption spectrometry based on the absorption of radiation emitted by the primary radiator by ground-state atoms. It is shown that the accumulation of radionuclides 137Cs and 90Sr, regardless of altitudinal zonality of the territory of Altai Republic, occurs weakly in meat products and has values significantly below the standards. In this regard, the territory of the Altai Republic is suitable for the production of environmentally friendly food. The different nature of HM accumulation in different altitude zones has been established. In the high-mountain zone, the content is minimal, which is probably due to natural factors. In the low-mountain zone, the role of anthropogenic factor increases, which leads to an increase in the content of heavy metals. The content of heavy metals is in the maximum permissible concentrations, in this regard, the research area is environmentally safe. The article provides an analysis of the practical experience of researching biogeochemical behavior of radionuclides, the data obtained are recommended to be used in monitoring biological objects of the environment.

Introduction

Despite its remoteness from industrial areas, the research territory of the Altai Republic is subject to anthropogenic influence. Geochemical processes occurring in the water–soil–plants–animals system play an important role in the migration of chemical elements since the water mass is the link between the source of pollution and their accumulation in soil horizons, plants. The quantitative content of radionuclides and trace elements makes it possible to assess in comparison with sanitary and hygienic standards and determine pollution degree. The purpose of the study is to identify patterns of distribution of radioisotopes and trace elements in biological chain soil–water–plants–animals depending on the altitude zonality of the Altai Mountains.

Materials and methods

The objects of the study were surface water, the most common soils, wild plants of the high, middle, and low mountains of the Altai Mountains, invertebrates, and the meat of domestic and wild animals.

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The method of gamma-spectrometric analysis was used, radionuclides ^{228}Ra, ^{232}Th, ^{40}K, ^{137}Cs were determined and studied on a spectrometric unit using Progress-2000 software and hardware complex;

The study of trace elements and heavy metals (Cu, Pb, Co, Mn, Fe) was carried out on QUANTUM-2 atomic absorption spectrometer by atomic absorption spectrometry based on the absorption of radiation emitted by the primary radiator by ground-state atoms.

Results and Discussion

The radionuclide migration system consists of the main components: "soil-water-plants-animals", the main links are: soil-plants, water-plants, plants-animals, and water-soil. Radionuclides along the "soil–plant–animal" chain enter the human body, accumulate, and harm health [1].

Migration also depends on the influence of factors on the system. Factors form two groups: the main and background factors that influence the system itself. The complex influence of factors determines the migration of radionuclides in the "soil-plants-animals" system.

Migration of radionuclides with soil is associated with their presence in the composition of "... of the known long-lived radioactive isotopes, ^{238} U, ^{232}Th, ^{40} K have the most geochemical value. Potassium-40 and members of two radioactive families originating from uranium-238 and thorium-232 belong to the main radioactive isotopes of the Earth's crust and are the main factors of natural radiation background" [2].

According to the data established by a group of scientists: "The background level in soil-forming rocks is 2-6 mg/kg for uranium, 8 mg/kg for thorium" [3] "Clark in soils is 3-5.1 mg/kg of uranium and 4-16 mg/kg of thorium" [4]. "The specific activity of ^{238} U, ^{232} Th, ^{40} K in black soils is 26, 44, and 500 Bq/kg, respectively. According to the data of the team of scientists of the Institute for Water and Environmental Problems of the Siberian Branch of the Russian Academy of Sciences (IWEP SB RAS), the average content of ^{238} U in Altai soils is 1.9±0.001, ^{232}Th – 6.0±0.003 mg/kg, ^{40}K activity – 419 Bq/kg" [2].

During the conducted studies, the effective specific activity was calculated (GOST 30108-94): $A_{eff} = A_{Ra} + 1.3_{ATh} + 0.09\,A_{K.}$

Total specific activity of natural radionuclides, determined taking into account their biological effects on the human body (Bq/kg) does not exceed its permissible value of 370 Bq/kg, the average values of Aeff, taking into account zonality, were: for the low mountains – 90.2, the average zone – 90.62, and high mountains – 92.9. The minimum values of total specific activity of natural radionuclides in soils of the Altai Mountains fall in May and early June (58.1-89.4 Bq/kg), the maximum falls at the end of July (86.2-110, Bq/kg). Mountain ranges are a natural factor for the accumulation of radionuclides. Foothill and low-mountain territories are characterized by a lower density of radionuclides, due to migration to deeper soil layers.

The spatial change in total specific activity of natural radioisotopes tends to increase from the plain to the mountainous territory (Fig. 1). The main part of radionuclides in soils is in 0-5 cm layer and their migration is very slow and depends on soil type. The migration of radioisotopes is influenced by the multifactorial nature of the soil composition (mechanical and mineralogical), as well as climatic conditions (temperature regime, precipitation, etc.).

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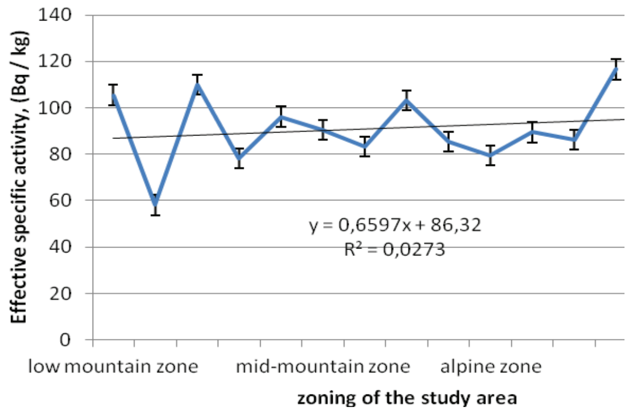

FIGURE 1. Spatial change of total specific activity and natural radionuclides of the Altai Mountains

To assess the differences between two independent samples according to the level of any radioisotope, the nonparametric method was used to identify differences in the parameter value between small samples. The calculations performed showed that median estimates are characterized by incomparably greater statistical stability than estimates based on arithmetic mean characteristics (Table 1).

TABLE 1. Statistical parameters of radionuclide content in soils of the Altai Mountains (Bq/kg)

Element	n	Min	Max	Std. Dev.	$X_{ave.}$	Mediana*	Abnormal values	Content according to literature data**
^{228}Ra	13	15.7	25.0	2.83	20.13	20.2	25.79	45-138
^{232}Th	13	24.0	42.0	6.53	30.72	27	43.78	44
^{40}K	12	320.0	470.0	40.19	371.67	360	452.05	419-500
^{137}Cs-	11	2.0	13.7	3.75	4.06	2.6	11.56	185−222

* Calculated background values of natural radionuclides in the studied samples;
** Background content according to literature data [2, 5-9].

As a result, background values of natural radionuclides were established in the study area, as well as abnormal jumps in content of some components in two altitude categories. For example, in one sample of the plain zone, the content of ^{228} Ra (25 Bq/kg) and at the testing point of the foothill part in 2019, the content of ^{37} Cs (13.7 Bq/kg) exceeds the average by the amount of two standard deviations.

The migration of radionuclides from soil to plants is influenced by some factors: contamination density, the forms of radionuclides in the soil, soil and climatic conditions of growing places, biological characteristics, and age of plants. With such a multifactorial nature, it is not always possible to establish the dependence of the intake of strontium-90 and cesium-137.

Uranium-238 and thorium-232, by having good mobility in soils [11], penetrate poorly into plants, which is characterized by their physiological unsuitability [12]. The insignificant content of cesium-137 in wild plants established by the authors [13] can be explained by both a low content in the soil and not high water migration. When analyzing samples of plants taken in the period from May to October 2019, a significant fluctuation in the content of cesium-137 strontium-90 in wild plants was found. The range of fluctuations in the values of the radionuclide activity content: cesium-137 ranged from 0.05 to 1.9 Bq/kg, and strontium 90 from 0.0-11.0 Bq/kg. The contents are in the category below the norm and do not pose an environmental threat.

Radium-226, thorium-232, and potassium-40 are absent in all plant samples.

When wild and domestic animals consume plant food or plant feeds, radionuclides accumulate, the shorter the food chain, the higher their level when ingested.

Cesium-137 accumulates in soft tissues, and strontium-90 is contained in bone tissue, which is excreted very slowly. Radionuclides affect all biological species regardless of their habitat and conditions of living [14]. In the

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meat samples of wild and domestic animals selected in June 2019, the following data were obtained: for ^{137}Cs less than 2 and ^{90}Sr less than 40, cesium-137 ranged from 0.04 to 1.1 Bq/kg, strontium 90 — from 4.0-26.0 Bq/kg. When calculating the radiation safety criterion (MUK 2.6.1.717-98), a hygienic assessment was obtained based on the results of measurements of specific activity of strontium-90 and cesium-137:

$$B = \left(\frac{A_{sp}}{H}\right)_{Sr} + \left(\frac{A_{sp}}{H}\right)_{Cr} = \left(\frac{26}{50}\right)_{Sr} + \left(\frac{1.1}{200}\right)_{Cr} = 0.5255 \quad (1)$$

– the actual and calculated values indicate radiation safety.

As a result of the study, the distribution of trace elements depends not only on the different physiological role but also on the ability of a natural object to absorb, accumulate or remove them from the system [15, 16].

The results of the study of HM content in the soil-water-plants-animals system are presented in Figures 2-5.

As a result of the study in the waters of the rivers of the Altai Mountains, the concentration range of metal forms has the form: Fe>Cu>Mn> Pb>Co. The content of gross forms of heavy metals in the surface waters of the middle-mountain part of Altai Republic was found to be lower than in the rivers of the high-mountain and low-mountain zones.

According to the results of the study, the dependence for iron, where the concentration increases with decreasing height: high mountains − 0.5963 mg/l; middle mountains − 0.6188 mg/l; low mountains − 2.0991 mg/l (Fig. 4).

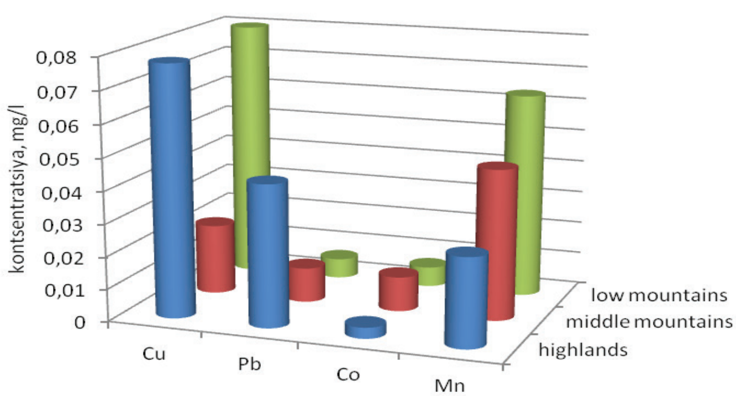

FIGURE 2. Trace elements and heavy metals in water of the Altai Mountains

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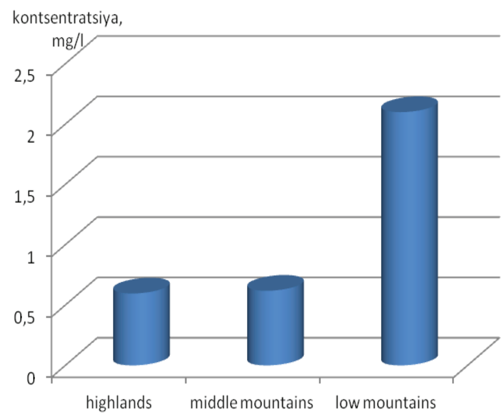

FIGURE 3. Content of gross iron in water of the Altai Mountains

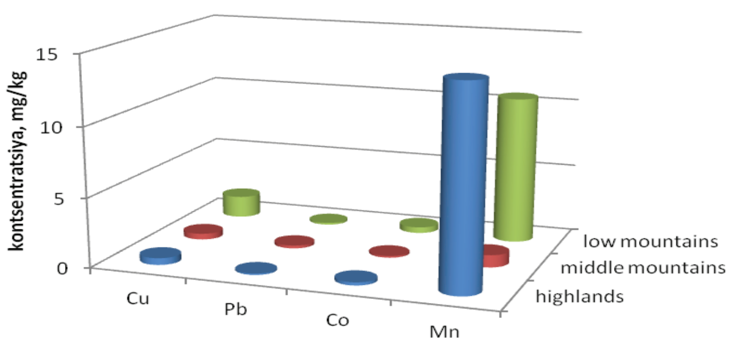

FIGURE 4. Trace elements and heavy metals in soil of the Altai Mountains

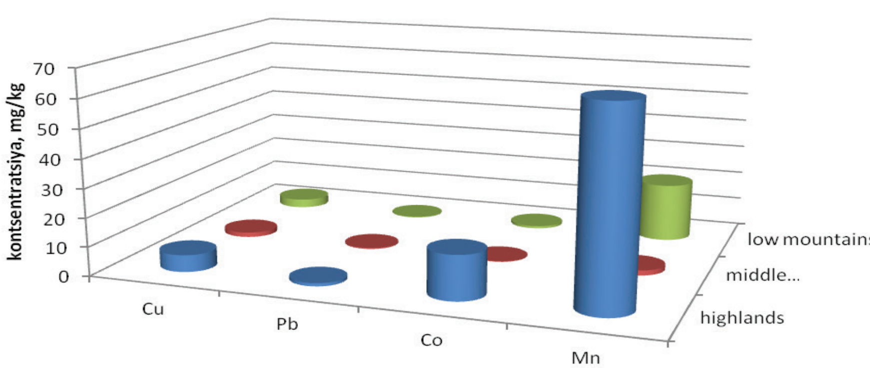

FIGURE 5. Trace elements and heavy metals in plants of the Altai Mountains

Fluctuations in manganese content in the studied soils are associated with content of slightly decomposed organic matter and coarse humus in the upper layers and had an acidic reaction of soil extraction. The highest values of manganese were recorded in the summer of 2019, and the maximum of iron was also recorded in the high-mountain zone (702.56 mg/kg). The maximum transition to a solution of manganese, presumably, will begin only after the removal of the bulk of iron, and then the remaining metals of copper, lead, and cobalt. Concentrations

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of studied chemical elements in soils of the Altai Mountains are considered background, these soils are not subject to either natural or anthropogenic pollution, except for areas of mercury ore occurrence.

In the aggregate of the studied wild plants from different altitude zones of the Altai Mountains. The average copper and cobalt contents are close to the background values, slightly higher than lead, and a relatively uniform distribution is typical for all plant species.

The analysis showed that all the studied plant species contain an increased content of iron and manganese heterogeneously − this is due to the presence of manganophile plants capable of concentrating manganese. Exceeding the maximum permissible concentrations was not detected.

The intake of trace elements directly in the plant-animal chain is related by the nature of their distribution in plants. The content of gross forms of lead in some invertebrate species of mid-mountain zone – 9.13 and low-mountain zone – 6.83 of the Altai Mountains, it turned out to be less than 5.27 mg/kg in the high-mountain zone.

According to the results of the study, there was no zonal dependence of iron accumulation: in low-mountains – 103.8 mg/kg, middle mountains – 52.90 mg/kg, low mountains – 200.6 mg/kg.

Lead content in animal organisms is the main criterion for determining the environmental safety of the territory in all studied samples, does not exceed the MPC value, and has a background nature of pollution.

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On the Use of the Robot Photographer in the Agricultural Segment of the Student Interdisciplinary Testing Ground

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Abstract. The article is devoted to the use of a project-based approach in the research and development work of students. The project realized by the students and employees of the robotics laboratory of Gorno-Altaisk State University (GASU) was developed using the method of project interfaces, which can be considered as one of the ways of project-based approach realization in the system of additional education. This work aims to investigate Robot Photographer, which is one of the design modules of the agricultural segment of the student interdisciplinary testing ground. It allows automating photo-reporting of the process of agricultural experiments and partially performs the functions of warning and protection of the object. In the introduction, the authors provide a background for the project-based approach application in the educational systems of different countries and give an overview of the implemented technical solutions aimed at automating greenhouse production using digital technologies. The systematic approach was used as a method of research in the interpretation and comparative analysis of the works of domestic and foreign authors. In the main part of the paper devoted to the description of the research results, the essence and technical characteristics of the Robot Photographer design module are stated. In conclusion, the authors describe the peculiarities of Robot Photographer operations, which could be observed during two seasons of its functioning as a part of the smart greenhouse control system included in the complex of student interdisciplinary testing ground.

INTRODUCTION

The project approach used in educational systems in various countries originated in the works of American educator William Kilpatrick, author of the article "Project Method" published in 1918 [1]. Now different versions of the project-based approach [2-4] are used depending on the educational technologies that teachers and educators use in their work. The experience of the authors of this paper shows that every educational project has a rather strong creative component, which is aimed at solving some real problem relevant to the end-user. One of such tasks set before the students and employees of the robotics laboratory of GASU was to automate agrotechnical (agricultural) experiments at the student interdisciplinary testing ground.

The considerable experience accumulated by different researchers in the field of automation of agricultural works was investigated during the preparation of this project. For example, the authors of the article [5] believe that the problem of food shortage in the world associated with climate change, which causes unpredictable weather conditions, can be solved by greenhouses. They do not require much space and allow maintaining a local internal microclimate. In [6] the authors describe a prototype of an intelligent sensor network, which has been successfully integrated with a WEB application and an automated control system for greenhouses, which allowed both local and remote control of the greenhouse through a standard WEB browser. The authors of the article [7] managed to develop a control system for the greenhouse on an Arduino Uno microcontroller, which should include servomotors pushing the roof when rain is detected. There is also a fan in the greenhouse, which turns on when the temperature is too high. Work [8] describes an automated greenhouse control system using Internet of Things (IoT) technology. Cloud services are used for remote monitoring and control of the greenhouse, which stores information and sends regular SMS messages to the farmer about the microclimatic conditions inside the greenhouse. An example of an

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automatic greenhouse control system based on an Atmega328 microcontroller and controlling four vital plant parameters such as temperature, light, moisture, and soil moisture is also given in [9]. Another example of the use of the IoT technology that controls parameters in a greenhouse is also given in [10]. It is controlled by six parameters monitored by six sensors: temperature, pressure, air humidity, soil moisture, light, and color. The difference between the technology presented in the article [11] and the examples described above is the control of carbon dioxide and other factors affecting plant growth. The authors of the paper indicate that LabVIEW was used to implement a user-friendly interface. The paper [12] also describes the application of the LabVIEW software platform for the development of an original hardware and software solution to simplify the development process of greenhouse automation systems. The Internet of Things is also used in the project described in [13]. The authors of the work note that they use the ThingSpeak cloud database to store information and a specially designed Web page is implemented to display the information. The project described in the article [14] was decomposed into a local part, implemented based on Raspberry PI 3 using the Python language, and a remote segment implemented as a website allowing the user to remotely interact with the greenhouse controller.

It should be noted that the works considered above were only about obtaining and storing information related to the control of the microclimate of the greenhouse, but working with photo and video materials recording the process of agricultural technological experiments (observation of plant growth, etc.) was never mentioned.

This work aims to study the Robot Photographer – one of the project modules of the student interdisciplinary testing ground. It allows automating photo-reporting of the agricultural experiments and partially implementing the functions of warning and protection of the greenhouse.

Materials and methods

During the creation of the paper, the authors used a systematic approach in carrying out phenomenological observations. The methodological basis of the study consists of methods of description, interpretation, comparison, problem, and comparative analysis of the works of domestic and foreign authors, as well as sources related to the technical documentation of the element base used in the implementation of the project.

Results

The student interdisciplinary testing ground [15, 16] is a rather complex technical system developed using the method of design interfaces [17], in which the agricultural segment occupies only a small part. Based on the fact that the financing of the project was carried out by the developers themselves, it was decided to implement the system as a set of DIY (Do It Yourself) modules. Experience has shown that when working in this mode it is convenient to use the method of project interfaces that allows decomposing the developed project solution into conditionally functionally independent subsystems – project modules. After decomposition, the resulting modules are described in terms of design interfaces, which are ways or protocols of interaction between modules used in debugging and the final assembly of the target project. Also, during the preparation phase, project micro-groups are formed from the project team members, corresponding, if possible, to the temperament groups described, for example, in [18].

Thus, in the design and implementation of the agricultural segment of the interdisciplinary student measurement site the “drip irrigation”, “microclimate monitoring”, “automated ventilation” and “Robot photographer” modules were identified.

The “Robot photographer” project module can be represented in the form of functional blocks, shown in Figure 1.

As mentioned above, module 7 is a conventional digital camera with a mechanical shutter. The automatic mechanical shutter was realized using the SG-90 servo drive, and the peculiarity of its realization was the space and time observance of the pushing force, which could be empirically determined during the experiments. Also, it became clear in the process of debugging the system that it was necessary to ensure that the camera was in the standby state, preventing the transition to the “sleep” and then to the “off” state. The issue with the mechanical blocking of the transition to sleep mode was also solved by an empirically determined series of soft presses on the mechanical shutter button, which kept the digital camera in standby mode. It was also planned to realize a rotary mechanism of aiming the Robot Photographer at the target in the future. The functioning algorithm of the design module was designed in such a way that different modes of photography (timed photography and unauthorized

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access photography upon sensors' triggering 8) were performed with sound accompaniment in the form of different melodies from children's cartoons, which were recorded into the MP3 player of sound message module 4. A general view of the prototype of the Robotic Photographer's design module is presented in Figure 2.

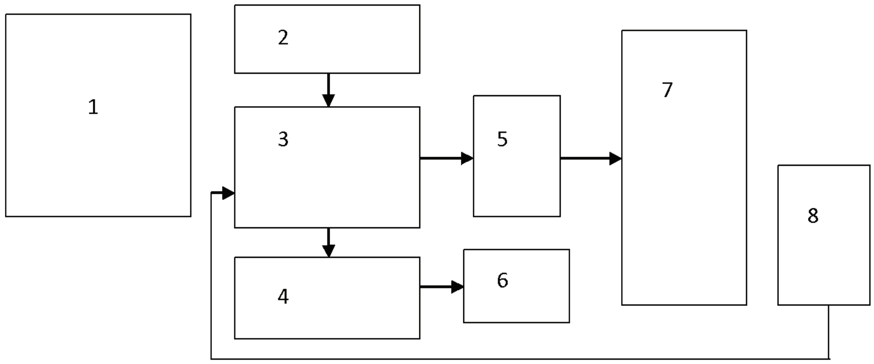

FIGURE 1. Flow diagram of the "Robot Photographer" project module: 1 – power supply unit; 2 – real-time clock; 3 – control microcontroller; 4 – sound message module; 5 – servo drive for pressing the shutter button of the digital camera; 6 – block of dynamic sound emitters; 7 – digital camera block; 8 – block of detection sensors

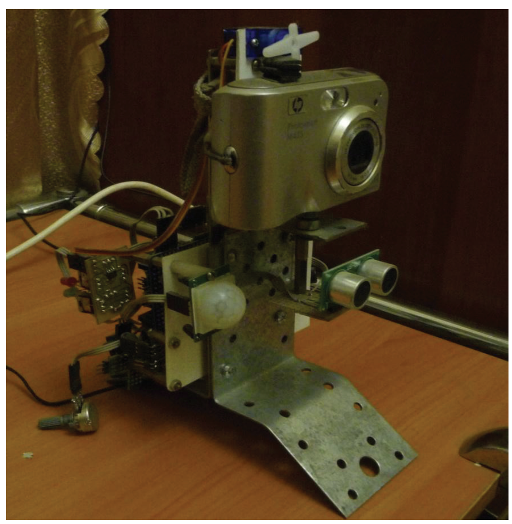

FIGURE 2. General view of the Robot Photographer

Figure 3 shows the Robot Photographer mounted in the corner of the greenhouse to cover the maximum viewing sector in the direction of the front entrance.

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FIGURE 3. Robot Photographer mounted in the corner of the greenhouse

The first tests of the Robot Photographer showed that if every trigger of the motion sensor is used for photography and intrusion warning, there is a large number of false “events” caused by, for example, the moving leaves of the cucumbers. To minimize false positives, a “repetitive interval check” algorithm was implemented, which highlighted a series of triggers at certain time intervals and only then gave the command to perform a photograph. Also, to save camera memory, a delay mode was implemented immediately after receipt of the signal from the sensors. To reduce the probability of a “missed trespasser”, the “interval” mode of photo-tracking was additionally introduced. In this mode, photos were taken both by triggering and by time at specified intervals. It should be noted that introduction of such a mode allowed receiving interesting photo-information about the life of plants in a greenhouse, especially at night. Quite interesting was an effect found when viewing night photos made in this mode. In the sequence of pictures, it was possible to observe how creeping stems of developing cucumber shoots rotate in one definite direction with a small frequency in search of support for pulling the shoot upwards. Since the process of changing the position of cucumber shoot creeping stems was very slow, it was impossible to observe it by the naked eye.

The opportunity to photograph the process of ripening of developing fruits from a single position appeared useful. It was interesting to discover the unevenness of fruit volume increase in different parts of the greenhouse, depending on the weather conditions outside the greenhouse and the state of the internal greenhouse microclimate.

The functioning of the Robot Photographer also allowed capturing interesting events related to the penetration of both people (in search of fresh cucumbers) and birds and cats into the facility, which were greeted by the robot photographer with musical compositions from children's cartoons.

Figure 4 shows the moment of the night operation of the Robot Photographer, recorded by the surveillance camera.

FIGURE 4. A moment of the Robot Photographer night operation

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Discussion

It is necessary to note the possibility of using pictures with date and time marks to compare growth rate and features of the development of cucumbers, tomatoes, and peppers in an experimental student's greenhouse while defining places of the most favorable dynamics of ripening of fruits as one of the essential results of the application of greenhouse Robot Photographer.

Conclusion

Thus, the Robot Photographer developed as a project module of the student interdisciplinary testing ground, allowed to observe and photograph the development of the plants planted in the greenhouse for two seasons, and also served as a passive warning and protection system.

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Technology for Early-Spring Potato Yields during Fall Planting

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Abstract. Potatoes are considered a frigolabile crop, so in early spring and late fall planting, their resistance to early-spring frosts should be increased. We propose an innovative resource-saving technology, using cryoprotectors, different mineral and organic fertilizers rates, and mulching for early potatoes. The article presents data on the increase of potato resistance to low temperatures, changes in the ratio of basic nutrients, increase in potassium, and the effect of cryoprotectors on the content of starch, sugar, protein, vitamin C and nitrate concentration in products. The most significant yield increases were obtained in variants with cryoprotectors, organic and mineral fertilizers during fall planting.

INTRODUCTION

The use of cryobiology achievements in agriculture has increased significantly in recent years [1-7]. Cryoprotectors are high-molecular-weight organic polymers, some of which are polyatomic alcohols by nature; they penetrate through the membrane and are a means of resisting crystallization of free water in the cellular fluid [8-11]. Because cryoprotectors do not have a toxic effect on the cell, in 1998, in cooperation with UNESCO, at the Ukrainian Scientific Research Institute of Cryobiology and Cryomedicine, a department of cryoprotectors was created. The institute is working on cryoprotective, physical, and chemical properties of various cryoprotective substances, properties of biological bonds, hydrophilic and hydrophobic balance, formation of cytotoxic and toxic bonds [12].

Potato is the most important food crop, the early harvest of which is in great demand in the food market. With the coronavirus pandemic, food security remains a significant concern. Specific climate conditions, early spring frosts, and summer droughts negatively affect the condition of early potato plantings and affect their yields. Under such conditions, introducing one of the innovative technologies, namely, the use of cryoprotective substances, changes the timing of planting and overcoming adverse climatic conditions.

In their research, H.N. Atabaeva, E.I. Koshkin, F.H. Hashimov, M.A. Hayitov, O.M. Nazarov, and T.F. Stribul noted that the range of -8 ºC to + 40-45 ºC on the upper or lower limit negatively affects the plants growth and development. If such temperatures persist for a long time, there is damage to vegetative organs and yield reduction. Potato tubers begin to develop at 7-12 ºC, grow rapidly at 20 ºC, and sprouts are severely damaged at 1-2 ºC [12-14].

Materials and Methods

Conducted field experiments and observations were based on generally accepted methods and recommendations of the Potato Research Institute, Methodology of Experiments in Vegetables, Horticulture, and Potatoes (2002). The object of the experiment is cryoprotectants, substances that increase frost resistance, mineral and organic fertilizers,

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and mulching. Different concentrations (propanediol-76) of 1.2-PD were studied. There were 9 variants in 6 replications on different nutrient backgrounds in the experiment.

Results

The effect of different concentrations of cryoprotectors, mineral, and organic fertilizers, mulching materials on increasing the resistance of early potatoes to low temperatures and product quality was studied. In the experiment (Table 1), potato tubers were planted in late fall and early spring, treated with an aqueous solution of cryoprotectors. Sprouting of potatoes in early spring was observed by variants. When planting in the fall, with the use of cryoprotectors and fertilizer sprouts were obtained by 5-11 days earlier than during regular spring planting, with the best performance – in variant 4, where 40 tons of manure and mineral fertilizers combined with cryoprotector 1.2 – PD in a concentration of 0.05%.

TABLE 1. Effects of cryoprojectors and optimal nutrition on the timing of potatoes vegetation and maturation (data for 3 years)

Variants of experiment	**Amount of fertilizer and planting time**	**Cryoprotectant concentrations, %**	**Planting time, day, month**	**Full sprouting, day, month**	**Sprouting, %**	**Top death phase, day, month**	**Early maturation relative to controls and variant 4, days**	**Growing period, days**
1	Control w/o fertilizer (fall planting)	-	18/11	18/04	35	07.07	- / - / 27	100
2	Control w/o fertilizer (spring planting)	-	06/03	20/04	92	06/07	1 / - / 26	97
3	40 t/ha manure +N_{180} P_{120} K_{60} (fall planting)	1.2-PD-0.01	18/11	03/04	86	20/06	17 / 16 / 10	96
4	40 t/ha manure +N_{180} P_{120} K_{60} (fall planting)	1.2-PD-0.05	18/11	29/03	96	10/06	27 / 26 / -	92
5	25 t/ha manure + N_{200} P_{160} K_{100} (fall planting)	1.2-PD-0.01	18/11	08/04	83	22/06	15 / 14 / 12	97
6	25 t/ha manure + N_{200} P_{160} K_{100} (fall planting)	1.2-PD-0.05	18/11	06/04	91	17/06	20 / 19 / 7	94

7	10 t/ha manure + N_{250} P_{180} K_{120} (fall planting)	1.2-PD-0.01	18/11	09/04	82	21/06	16 / 15 / 11	98
8	10 t/ha manure + N_{250} P_{180} K_{120} (fall planting)	1.2-PD-0.05	18/11	10/04	89	16/06	19 / 18 / 6	96
9	N_{200} P_{160} K_{100} (fall planting)	-	18/11	20/04	92	04/07	3 / 4 / 24	98

A similar situation was observed in other variants, however, the concentration of 1.2-PD-0.01% was less effective than that of 1.2-PD-0.05, and even short-lived low temperatures in early spring did not damage sprouts.

Potato sprouts in all variants developed depending on the nutrients applied to the soil, mulching, soil moisture and temperature. Potato growing season depends on the variety, and in the experiment – depending on the technology in different variants, thus, in the control variant lasted 97-100 days, and in the variant 40 t/ha of manure + $N_{180}P_{120}K_{60}$ (autumn sowing) + option 1.2-PD-0.01% – 96 days. In this variant, the phase of haulm dying down was observed on June 20, and ripening was 10 days earlier than the fall control and 6 days earlier than the spring control. In contrast, in variant 40, t/ha of manure + $N_{180}P_{120}K_{60}$ (autumn sowing) + 1.2-PD-0.05% full ripening came 20 days earlier than the fall control and 16 days before the spring control [15].

Depending on the degree of protection of seed tubers of potatoes from low temperatures, the stem and root systems are better formed. The plant is more resistant to external factors during the growing season, better absorbs nutrients, the interphase period shortens, and this results in higher yields. The most significant yield increases were obtained in variants with cryoprotectors, organic and mineral fertilizers during fall planting. In variant 4-6, the increase to the spring, conventional planting was 16.9; 12.1 t/ha respectively.

Potato tubers obtained in the experimental variants did not differ from the spring sowing, and for some indicators, such as starch content of 14.1-14, 3 %, sugars 0.61-0.84 %, vitamin C 17.5-18 mg/%, protein 1.82-1.90 %, and in some variants even exceeded (Table 2). It was found that the variants with fertilizers and cryoprotectors have 0.8 % more starch, 0.11 % more sugar and 0.05 mg more vitamin C than the control. In the variant 40 tons of manure + $N_{180}P_{120}K_{60}$ (fall seeding) + 1.2-PD-0,05% starch content of 15.4%, sugar content of 0.76%, vitamin C 18 mg, protein – 1.84%. These characteristics are 1.2% higher for starch and 0.15-0.02% higher for sugars than for control. Nitrates in some fertilized variants were slightly higher than in control.

TABLE 2. Yield and tuber quality

Variants of experiment	**Amount of fertilizer and planting time**	**Concentration of cryoprotectors, %**	**Yield capacity, t/ha**	**Chemical composition of potato tubers, %**				
				Starches, %	**Sugar, %**	**Vitamin C, mg**	**Protein , %**	**Nitrate s NO_3^- mg\kg**
1	Control w/o fertilizer (fall planting)	-	10.93	14.1	0.61	17.5	1.82	150
2	Control w/o fertilizer (spring planting)	-	13.36	14.3	0.84	18	1.9	146
3	40 t/ha manure +N_{180} P_{120} K_{60} (fall planting)	1.2-PD-0.01	17.65	14.2	0.76	18	1,8.	142
4	40 t/ha manure +N_{180} P_{120} K_{60}	1.2-PD-0.05	30.25	15.4	0.76	18	1,8.	140

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	(fall planting)							
5	25 t/ha manure + N_{200} P_{160} K_{100} (fall planting)	1.2-PD-0.01	16.68	14.2	0.72	18	1.84	145
6	25 t/ha manure + N_{200} P_{160} K_{100} (fall planting)	1.2-PD-0.05	25.48	14.9	0.72	18	1.86	155
7	10 t/ha manure + N_{250} P_{180} K_{120} (fall planting)	1.2-PD-0.01	16.68	14.1	0.83	18.4	1.9	160
8	10 t/ha manure + N_{250} P_{180} K_{120} (fall planting)	1.2-PD-0.05	20.85	14.6	0.84	19.1	1.98	158
9	N_{200} P_{160} K_{100} (fall planting)	-	13.35	13.8	0.72	18	1.84	145

Discussion

The possibility of early planting with the use of cryoprotectors and the effect of potassium fertilizers on the frost resistance of potatoes allows for an early harvest, which is in demand in the market. At the same time, the problem of the effect of cryoprotectors on product quality has been studied, and no residuals have been found. All these data were obtained for the first time and are consistent with the materials of studies carried out on other crops [13, 16].

Conclusion

The application of mineral and organic fertilizers and treatment of potato tubers with cryoprotectant improves plant nutrition and creates better conditions for the harvest of early potatoes of better quality, which leads to the population provision with early potatoes, whose price in the market can significantly increase farmers' income.

In a pandemic, growing early potatoes with new, innovative technology is a requirement of the times.

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The Interdependence of the Humus State and the Productivity of Individual Crops on Irrigated Soils

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Abstract. The results of studies of changes in the humus state and nutritional regime of soils in the serozem belt and the desert zone of the Zarafshan Valley under the influence of anthropogenic factors, such as irrigation and individual agricultural techniques in cultivating agricultural crops, are presented. The results of field route studies, stationary field experiments, and laboratory studies on the study of agrochemical characteristics, the processes of changing the group and fractional composition of humus of different types of soils located at different heights above sea level under different plant associations and agricultural crops, are given. The relationship between groups and fractions of humus along the soil profile and the correlation with soil nutrients are studied in detail. The recommendations are given for increasing the productivity of soils and the effectiveness of using mineral fertilizers.

INTRODUCTION

In the conditions of the Zarafshan Valley, as a result of erosion processes with improper irrigation and depending on the genesis of the soils, groups of soils with different content and quality of humus have appeared. An increase in anthropogenic influence on the soil cover leads to a decrease in the humus content, a change in the ratio of humic and fulvic acids, as well as a significant change in the fractional composition of humus.

The research aimed to establish the regularities of changes in the humus state and nutrient regime of soils in the serozem belt of the Zarafshan Valley under the influence of irrigation and agricultural crops.

For the first time, the study of the influence of soil fertility in the Zarafshan Valley, differing in soil formation factors, on various agricultural crops was conducted. The influence of the prescription of development (irrigation) and agricultural technology of cultivating agricultural crops was established on the qualitative composition of humus, yield, and product quality.

Materials and methods

The soil samples and their preparation for analysis were conducted by the generally accepted method. The content of mobile forms of nitrogen was determined in fresh soil samples. The remaining parameters were determined in dried samples.

The content of humus was determined by the Tyurin method and Nikitin's modification; the fractional and group composition of humus was determined by the Tyurin method and the modification by Ponomareva and Plotnikova. In assessing the quality of humus, the authors adhered to the system of indicators developed by L.A. Grishina and D.S. Orlov.

The accounting of yield and statistical processing was conducted according to Dospekhov. Nutrient removal and fertilizer utilization coefficient correspond to generally accepted methods.

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Results

Numerous experiments conducted in various soil and climatic conditions show that irrigation for 42 years in Ukraine has led to a decrease in the humus content in 4450 observation sites [1]. In 26 experiments, the USA [2] show that the application of fertilizers without taking into account the state of the soil leads to irrational use of fertilizers.

The study of the content of humus and its fractions in an arid climate is of particular importance, since humic substances contribute to conserving moisture in the soil [3].

It is known that the state of humus in the soils of the Zarafshan Valley is affected by climate conditions and agricultural practices of the main crops [4-9].

Based on the study of the fractional composition of soil humus in the middle of the 20th century [10, 11], a nomenclature of fulvic and humic acids, as well as humins was created. This classification is based on their solubility in the aquatic environment, however, fulvic acids are soluble in acidic and alkaline environments, humic acids – in alkaline one, and humins are insoluble in any environment.

Thus, the humus of all types of the Zarafshan Valley can be attributed to the fulvate type, and in the upper horizons, to the humate-fulvate type, when the C_{ha}:C_{fa} ratio is in the range of 0.5-1, in deeper horizons it becomes fulvate-humate, i.e. C_{ha}:$C_{fa}< 0.5$.

Depending on the cultivated crops and mechanical composition, the content of humic acids from the total amount of humus varies, their amount is greater against the background of alfalfa and in the garden. Thus, when cultivating alfalfa, the content of humic acids in the 0-30 cm layer was 24.9%, in the 30-50 cm layer – 23.5%, and after many years of wheat cultivation – 20.9% and 20.1%. Down the soil profile, the content of humic and fulvic acids decreased, especially the humic acids.

In the subregion of the plains of the serozem belt, the content of humic and fulvic acids, depending on the horizon, soil type, as well as cultivated crops, varied widely. A sharp decrease in the content of both humic acids and fulvic acids is seen along the soil profile, and this is especially clearly observed in humic acids.

Hydromorphic soils contain more humic acids than semihydromorphic and automorphic soils, and their content decreases down the profile.

In the newly developed gypsum-bearing light serozems, the content of humic and fulvic acids in absolute quantity and relative content is less than in other considered soils. Thus, humic acids were 16.5 in 0-30 cm, and 14.2% in 30-50 cm soil layers. There were more fulvic acids, amounting to 24.8% in 0-30 cm and 23.7% in 30-50 cm soil layers. The content of humic acids down the soil profile varied within a wider range.

In the desert zone of the Zarafshan Valley, the content of humic acids in hydromorphic soils was very low compared to similar soils of the serozem belt. Thus, in the newly developed meadow soil in the 0-30 cm layer, the content of humic acids was 10.1%, in the newly irrigated soil – 11.6%, in the old-irrigated soil – 15.4%, and in accordance with the prescription of these soils, the decrease in the proportion of humic acids in the composition of humus was more uniform. The proportion of fulvic acids changed in the same pattern, and the proportion of non-hydrolysable humus residue was contrary to that. Thus, the content of humic acids is higher in the old irrigated meadow soil.

In the meadow-oasis soil of this region, in the 0-30th layer of the soil, humic acids accounted for 20.3% of the total soil carbon, in the 30-50 cm layer – 18.8%, and fulvic acids, respectively, 27.7% and 26.3%, that is, the content of humic, and especially fulvic acids, was higher than in meadow soils. The content of humic and fulvic acids decreased down the profile, and especially after tilled crops.

In all soils of the desert zone, the content of humic acids changes during the cultivation of alfalfa, and this was especially noticeable in the upper soil horizons. When cultivating wheat, corn and cotton, the lowest content of humic acids is noted.

Thus, from the west to the east of the Zarafshan Valley, in the soils similar in type, the content of humic acids decreases, while the content of fulvic acids does not change significantly. When cultivating alfalfa and in gardens, the content of humic acids increases, and after tilled crops and wheat – it decreases.

In automorphic and semihydromorphic soils of the Zarafshan Valley, the increase in the proportion of non-hydrolysable substances down the profile occurs mainly due to a decrease in the content of humic acids.

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For all the soils of the Zarafshan Valley, the content of 2 fractions in the composition of humic and fulvic acids was the highest. The exception is the virgin typical serozem of the sub-region of the foothills, where the proportion of the 1st fraction of fulvic acids somewhat exceeds the second. The share of the 1st fraction in the humus of virgin serozem is greater than in the old irrigated analog, but, decreasing along the profile of virgin soils, it approaches the content of this fraction in the old irrigated typical serozem.

When cultivating alfalfa, the absolute content of the 1st fraction of humic acids increased, while when cultivating wheat and tobacco, on the contrary, it decreased. The content of the 2nd fraction was also high while cultivating alfalfa and under the apple orchard. The cultivation of wheat led to a decrease in this fraction. The content of the 3rd fraction of humic acids, less mobile, was higher while cultivating these crops (Fig. 1, 2).

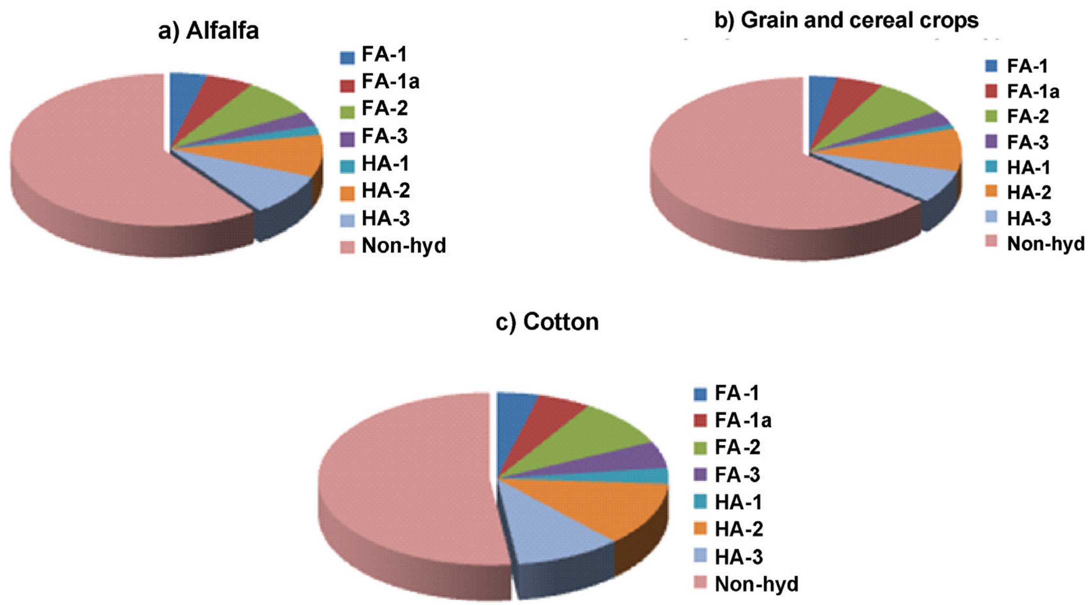
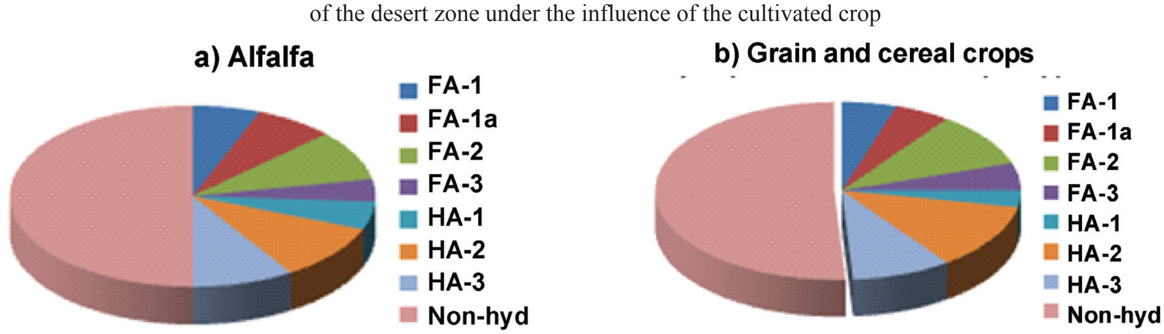


FIGURE 1. Changes in the fractional composition of humus in the upper (0-30 cm) horizon of the newly irrigated meadow soil of the desert zone under the influence of the cultivated crop



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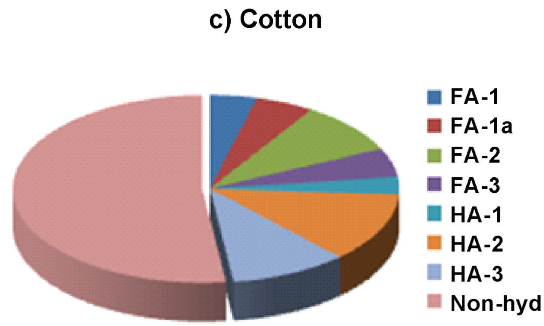

FIGURE 2. Changes in the fractional composition of humus in the upper (0-30 cm) horizon of the anciently irrigated meadow-oasis soil of the desert zone under the influence of the cultivated crop

The content of the 1st fraction of humic acids was higher in meadow soils, and it increased with the prescription of irrigation. The content of fractions 2 and 3 changed in exactly the same order. The results show that the relative content of the 3rd fraction of humic acids is higher on typical serozem. The absolute content of all fractions down the profile decreases, but the relative carbon content of humic acids varies in different ways. Thus, the content of fractions 1 and 2 in humic acids decreases down the soil profile, while the content of the 3rd fraction, on the contrary, increases. With the weighting, the mechanical content of all fractions of humic acids increases.

In the newly irrigated typical serozem, in the upper layers, the content of fractions 1a and 1 is greater than the content of fraction 3, and vice versa in the lower layers. Fulvic acids are containedin the upper layers, mainly in the form of the 2nd fraction, and in the lower –in the form of the 3rd fraction. A decrease in the fractions 1a and 1 of fulvic acids as the genetic horizons deepen, is apparently due to a decrease in the activity of microorganisms, as well as a decrease in organic residues.

In the soils of the serozem belt, meadow soils have the most optimal fractional composition when cultivating alfalfa and under perennial plantings. Cultivation of tilled crops such as cotton, tobacco, corn, as well as grain crops negatively affects the fractional composition of humus.

In the desert zone of the Zarafshan Valley, on the anciently irrigated meadow-oasis soil, given a light granulometric composition of the soil, the content of fraction 1 was greatly reduced, and therefore it was very low in sandy and sandy-loam soils. In sandy and sandy-loam soil horizons in terms of the mechanical compositions, the content of the 2nd fraction of humic acids is greatly reduced, which is associated with a low content of exchangeable calcium in these soils. The content of the 3rd fraction was relatively high, and the change in the content of the 3rd fraction of humic acids along the profile in the anciently irrigated meadow-oasis soil is not observed.

The relative proportion of fractions 1 and 3 in the composition of humic acids increased and the proposition of the 2nd fraction decreased down the soil profile. It means the change in the qualitative composition of humus, depending on the cultivated crops. Alfalfa increased the contents of all fractions of humic acids, especially in the upper soil layers (Figs. 1, 2).

The content of the fraction 1a down the soil profile decreased, but it was more than the content of fractions 1 and 3, and less than the 2nd fraction. Contents of the fractions 1a, 1, 2 in the soil profile decreased, and the 3rd fraction increased. The process of forming humic substances is undergoing in the upper horizons, so the content of the fractions 1 and 1a here in the absolute and relative amount was more than in the lower horizons. In the lower horizons, the content of 1 and 1a fractions in absolute and relative amounts is less, and therefore the proportion of the 3rd fraction in the lower horizons is greater.

The authors' studies on observational plots located on different types of soils in the Zarafshan Valley showed a clear dependence of crop yields on the genetic properties of soils, humus content and mechanical composition. The effectiveness of the applied fertilizers also depends on these indicators. This is consistent with the results of many studies [12-14] and does not correspond to the opinion of some researchers [15].

Cultivating agricultural crops on meadow soils (Table 1), containing more humus, gives a greater effect from the applied fertilizers than on meadow-serozem and typical serozems.

TABLE1. Efficiency of usingfertilizers in the soils of the Zarafshan Valley

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Observation site number	Soil type	Mechanical composition	Crop	Yield c/ha	Yield increase due to fertilizers, c/ha	Humus content, in %	The correlation of C_{ha}: C_{fa} of humus	Coefficient of using fertilizer nutrients in plants, in %.		
								N	P_2O_5	K_2O
22-K	Typical serozem	medium clay	Cotton	32.2	18.7	0.84	0.9	35.9	18.0	76.4
23-K		light clay		26.5	13.7	0.64	0.7	30.2	16.5	68.5
18-U		medium clay-loam	Wheat	42.0	26.4	0.86	0.9	42.6	22.0	73.9
7-U		light clay-loam		38.5	23.4	0.78	0.7	34.7	19.7	67.1
3-U		heavy clay-loam	Alfalfa	142.0	87.0	1.46	1.1	-	19.8	79.2
3-U		light clay	Apple tree	132.0	101.0	1.31	0.8	23.4	23.1	50.5
41-K	Meadow -serozem	medium clay	Cotton	33.7	20.2	0.83	0.7	43.0	18.7	78.5
16-K	Meadow soil	medium clay-loam	Cotton	35.8	21.5	1.05	0.7	44.7	18.8	79.7
8-K		medium clay	Wheat	44.6	28.4	1.26	0.9	45.1	23.1	78.3
3-K		light clay	Apple tree	105.0	77.0	1.14	0.8	20.8	18.0	47.7
3-N	Light serozem	light clay	Alfalfa	125.0	74.0	0.77	0.6	-	19.5	60.1
17-N		light clay	Apple tree	76.0	51.0	0.98	0.7	15.0	14.0	34.2
231-G	Newly irrigated meadow soil	heavy clay-loam	Cotton	22.0	9.9	0.86	0.4	26.4	12.7	53.5
367-G		light clay-loam	Wheat	22.8	8.6	0.76	0.6	17.9	8.7	27.5
333-G		light clay-loam	Alfalfa	106.0	56.0	0.79	0.8	-	17.2	58.5
131-V	Old-irrigated meadow soil	light clay	Cotton	28.5	15.4	1.21	0.5	33.1	18.0	73.4
109-V		heavy clay-loam	Wheat	32.5	17.1	0.95	0.6	27.4	14.6	51.5
27-V		medium clay-loam	Alfalfa	118.0	63.0	1.18	1.0	-	18.2	64.3
200-V	Meadow -oasis soil	light clay	Cotton	30.8	17.1	1.35	0.8	35.1	18.7	76.0
315-G		light clay	Wheat	36.5	20.6	1.23	0.8	32.2	17.1	60.2
119-V		light clay	Alfalfa	136.0	77.0	1.11	0.9	-	25.0	74.3

Discussion

As far as all types of soils in the Zarafshan Valley are concerned, it can be noted that the qualitative composition of humic acids was the best in typical serozems and, to some extent, in meadow-serozem soils. In the newly

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developed, newly irrigated, especially the meadow soils of the desert zone, the light serozem of the serozem belt, the content of all humic acid fractions was very low, especially the 1stfraction.

A long-term permanent cotton crop, wheat cultivation and, in fact, a two-yield crop have significantly reduced not only humus reserves, but alsothe ratio of C_{ha}:C_{fa} in favor of an increase in fulvic acids. In addition, the proportion of non-hydrolysable residue noticeably increased. Long-term irrigation, especially on soils with a not very thick humus horizon, leads to a change in the qualitative composition of humus, increasing the proportion of non-hydrolysable residue.

In all types of soils, there is a lack of organic matter input, which leads to a decrease in the proportion of fractions 1 and 2 of humic acids and the fractions 1a, 1, 2 of fulvic acids and a rather noticeable increase in the proportion of fraction 3 of these acids.

Maintaining the balance of humus and some improvement in the ratio of fractions of humic and fulvic acids in a positive direction is observed only while cultivating alfalfa and perennial plantations.

Conclusion

In the process of long-term irrigation and agricultural use in old- and anciently irrigated soils, organic matter accumulated, not only in the upper layers, but throughout the entire soil profile. Along with the increase in the content of humus, as the duration of irrigation increases, its quality improves. By increasing the proportion of all fractions of humic and fulvic acids, the proportion of non-hydrolysable residue in the humus composition decreases.

The effectiveness of mineral fertilizers, the removal of nutrients, the coefficient of use of mineral fertilizers and crop yields depend on the type, genesis of soils, climatic conditions, and humus content in the soil. The rational use of fertilizers, taking into account these factors, including the humus state of the soils of the examined region, firstly, will reduce the costs associated with their use, secondly, help improve the quality of agricultural products, and thirdly, get additional food products by increasing the yield of agricultural crops, in particular, obtaining an additional yield of raw cotton 2-2.5 c/ha, grain crops 4-4.2 c/ha, fruit crops 10-12 c/ha, as well as fodder crops 20-25 c/ha. Therefore, it will enable to additionally obtain finished food products from 1 hectare: 18-22 kg of cottonseed oil, 280-300 kg of flour, 1-1.2 tons of fruit, and 900-1200 fodder units or 200-250 kg of digestible protein to increase livestock production.

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Thermal Regime at Flowing Around the Plate with Bulk Media

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Abstract. At the moment, the thermal regime of metal plates streamlined by bulk materials is not sufficiently studied. The humidity dependencies of bulk materials in process of cooling metal plates are not clearly defined. A universal efficiency criterion for cooling in bulk media is not developed. The research aims to study the thermal regimes of a metal plate with various gravity-moving bulk media. To determine the relation between cooling by bulk media to mass flow rate and humidity of bulk media. To deduct the criteria of effectiveness of cooling by bulk media. The next methods were used: registration of temperature changes in plate and bulk medium in the gravity moving process of bulk media on the plate, flow rate of bulk media registration. This paper presents an original experimental installation for studying heat and mass transfer processes in bulk media. A series of experiments were carried out. A method for evaluating the effectiveness of cooling solid surfaces by streamlining it with bulk media was proposed. Dependences of the parameter, that characterize the cooling efficiency of solid surface flowed on by the bulk media to the flow rate and on the humidity content of a granular medium were deducted. A cooling effectiveness comparison between spilling various bulk media (salt, soda, semolina, millet and building sand) and water on the solid surface was made. A study was carried out to determine the presence of a correlation between the cooling efficiency and the value of the thermal diffusivity for the tested media.

INTRODUCTION

Heat transfer during gravity-moving bulk media interaction with hard surfaces occurs in different sectors of economy. In the food industry – when loading grain mass for storage or transportation, groat, flour, or other ingredients. In various technological processes – agriculture, construction, mining, metallurgy, chemical industry, energy and transport.

The issue of studying the outflow of bulk media as such isn't new – many works have been dedicated to it in the last century [1]. Among others, our team was engaged in the study of gravity–moving bulk media [2]. All over the world: in all climatic conditions, the issue of studying granular media is relevant [3]. As a rule, in particular applied cases, the data constancy of operating parameters for various technological processes, equipment reliability and safety issues are of interest. As for heat and mass transfer with the participation of bulk media, first of all, the most important part is the integrity of that substance [4-6]. At the same time, the issues of heat transfer between gravity–moving bulk media and solid surfaces are ignored. Although various works in this direction have appeared in recent years [7-13]. The recorded dynamics of the phenomenon indicate the occurrence of self-heating in two stages: a slow temperature increase and relative humidity in intergranular spaces; and an accelerated temperature rise along with a slight increase followed by a decrease in relative humidity [14].

The tasks of heat and mass transfer in bulk media are important and not always clearly calculated theoretically. In bulk media, prone to self–heating, various processes occur simultaneously [6]. Moreover, some of them compete with each other – they consume the same thermal energy, and therefore suppress each other [15]. And some, on the

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contrary, are mutually generative. For example, in grain mass, a slight temperature increase leads to the formation of comfortable conditions for insects development. The rapid growth of insects, accompanied by their active respiration, causes an increase in humidity, which in turn leads to developing mold [16-18].

However, biological components are quite diverse and simultaneously depend on different factors, which in turn can be both interrelated and independent of one another [19]. Microbial and chemical heating occurs at lower and higher temperatures, respectively. Moisture control is an effective measure to reduce self–heating [18, 19].

The above–mentioned self–heating process in different granular materials manifests itself in different ways. Self–heating of barley or wheat is characterized by different parameters values than the same process in legumes or oilseeds [16]. For moderate external heating temperatures, measurements show that the conversion process is globally exothermic [20]. The situation is also different for a handful and a large heap of bulk media [21]. And if we consider the self–heating of coals and rocks [17, 22], then with the apparent similarity of the phenomena, the differences in the physical and chemical processes taking place will be even more significant.

Therefore, for the development of universal mathematical models, it is crucial to obtain and study the reference patterns. On the basis of these patterns, we complete the specifying details that characterize the features of a particular task. This paper aims to fill the gap in the accumulation of experimental data on heat transfer between an inclined metal plate and gravity–moving bulk media. And more specifically, the study of the patterns of gravity–moving bulk media along a heated inclined metal plate for subsequent generalization, which has both fundamental importance and practical interest in various applied problems.

Materials and methods

As noted in the introduction, this paper aims to experimentally study heat transfer between an inclined metal plate streamlined with gravity–moving bulk media. Of greatest interest is the connection between the mass flow rate of a bulk medium and a change in the surface temperature which it streamlines.

Mathematical formulation of the problem

Starting from the century before last (or the 18th century), there has been a tradition of using empirical formulas in engineering practice in the form of the relation between dimensionless similitude criteria. It should be noticed that it is very convenient for solving many practical problems. However, this approach leads to the uniqueness of the constant coefficients specified for certain specific conditions, which imposes certain restrictions. For example, the use of various media: equations that fairly describe some substances' behavior are inapplicable for the description of other substances. It seems logical to us that for the construction of universal models one should rely on fundamental relations. In our case, this is the non–stationary heat conduction equation for a solid medium:

$$\frac{\partial T}{\partial \tau} = a\left(\frac{\partial^2 T}{\partial x^2} + \frac{\partial^2 T}{\partial y^2} + \frac{\partial^2 T}{\partial z^2}\right) = a\nabla^2 T \qquad (1)$$

where T – temperature, ºC; τ – time, s; x, y, z – spatial coordinates, m; a – the thermal conductivity, m^2/s [23].

Selection of bulk media

It is logical to consider (and compare) the behavior of the thermograms of the heated surface when interacting with various bulk media used in various sectors of the economy. For the experiments, we chose semolina, millet, edible salt, soda, construction sand. In bulk media that are food products, with a change in temperature and humidity in the process of heat and mass transfer, irreversible changes of a biological nature occur. Therefore, building sand was chosen as a standard substance, since its properties after repeated heating are unchanged – in cooling and moistening – drying cycles. In addition, along with bulk media, experiments were carried out on the water in the droplet phase to compare the heat transfer characteristics of bulk media with some standards.

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Experimental research technique

In various practical situations, the streamlined surfaces can be of different shapes and made of different materials (steel, plastics, concrete, graphite, etc.). As a starting point (the so–called specified experiment) considering the heated surface, the simplest case – a single flat plate was chosen. Moreover, it makes sense to consider a copper plate, since, copper is widely more studied material with known properties, with high thermal conductivity, which is very convenient for research goals. In the experiments, the working section was a rectangular gutter with a flat width base of 64 mm, a height side of 22 mm, and a length of 600 mm, bent from a copper plate 0.5 mm thick.

The flow was carried out due to the gravity–moving bulk media. For this, the copper plate was posed so that one edge of its working surface (64 mm long) was horizontal, and the other (600 mm long) was at a given angle to the horizon. Observation of cooling is most evident by recording thermograms. For this purpose, in the center of the heated zone of the chute plate temperature was measured. The volumetric flow rate was also set and the mass flow rate of the bulk medium was measured. In addition, the initial temperature of the bulk medium and the temperature of its free surface during flow along the chute were recorded.

Experimental setup

There are various experimental means and methods for studying heat and mass transfer in bulk media [24-32]. However, they are all designed to work with stationary bulk media. Therefore, in this paper, it was decided to create our unique experimental setup. A schematic of our experimental setup used in this work is shown in Fig. 1.

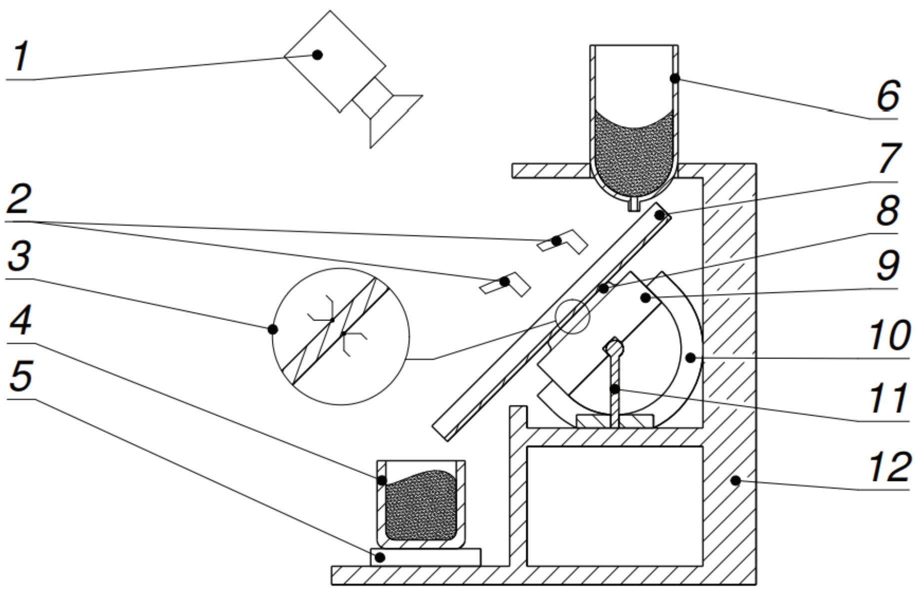

FIGURE 1. Schematic of an experimental setup for studying heat and mass transfer in a gravity–moving bulk media over a hard surface: 1 – video recording equipment, 2 – pyrometers, 3 – thermocouples, installed on opposite sides of the streamlined plate, 4 – receiving hopper 5 – scales, 6 – hopper with the original bulk medium, 7 – working section, 8 – heating element, 9 – heating element control unit, 10 – system of spatial positioning of the working area, 11 – tested section fixing, 12 – support structure.

Experimenting

The algorithm for the experiment was as follows. The temperature and humidity in the laboratory room and in the studied bulk media were recorded. The bulk medium under study was weighed, that it was loaded into hopper 6. The measurement results were recorded in the protocol. The recording was started by video recording equipment 1. The bulk medium from bunker 6 was fed to the working section 7, then into the receiving hopper 6, fixed on the scales 5. Temperature recording devices (pyrometers 2 and thermocouples 3) recorded the measured values. According to the scales readings 5 and the timecode from the experiment video, the instant and integral flow rate of

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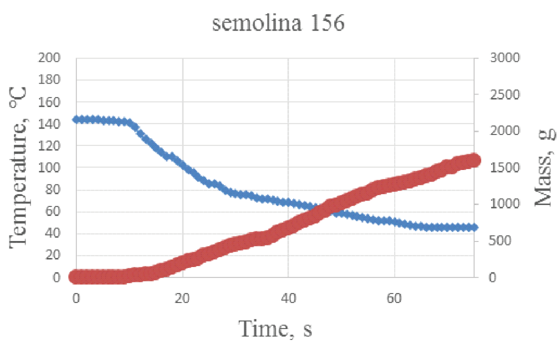
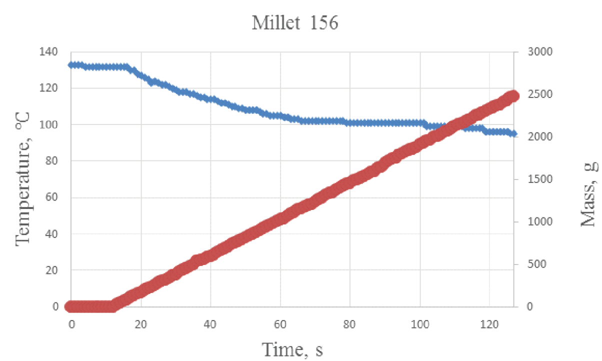
the bulk medium was determined. The video recording was stopped. The readings were used to determine the correction for the heating of the working section by gravity–moving bulk medium due to friction. The bulk medium was re-weighed. Based on the difference in the mass values of the test medium before and after spilling, the amount of dispersion was calculated, which was taken into account when determining the gravity-moving bulk medium through the working section. The bulk medium from the receiving hopper 4 was removed into hopper 6. The heating element control unit 9 was switched on. It was expected when the temperature of the working section 7, which was in thermal contact with the heating element 8, reached a stabilized value. The temperature and humidity were measured again in the laboratory room and in the bulk medium under study. The recording was started by video recording equipment 1. Spillage of the bulk medium began and all the previously described procedures were repeated.

Results and discussion

As a result of the experiments, data sets were obtained, the examples of which are shown in Fig. 2.



a)



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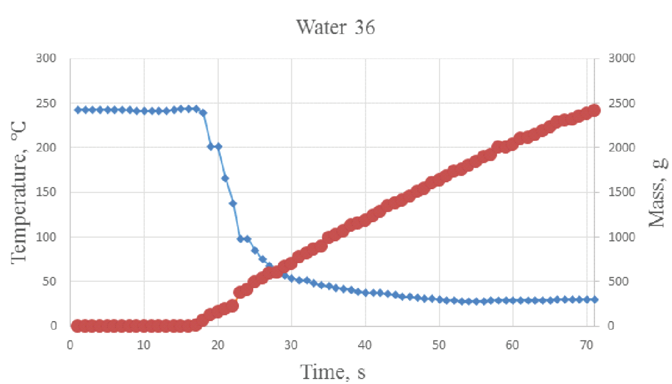

c)

FIGURE 2. Synchronous dependences of the temperature of cooled surface and the instantaneous mass flow rate of some tested substances (♦ – Temperature, ºC; ● – Mass of bulk media, g): a – semolina, b – millet, c – water

As expected, with an increase in the intensity of the interaction of a bulk medium with a streamlined surface, the surface cooling rate should increase. Figure 3 shows the thermograms of cooling a flat copper plate when interacting with dry sand moving along it by gravity for different flow rates.

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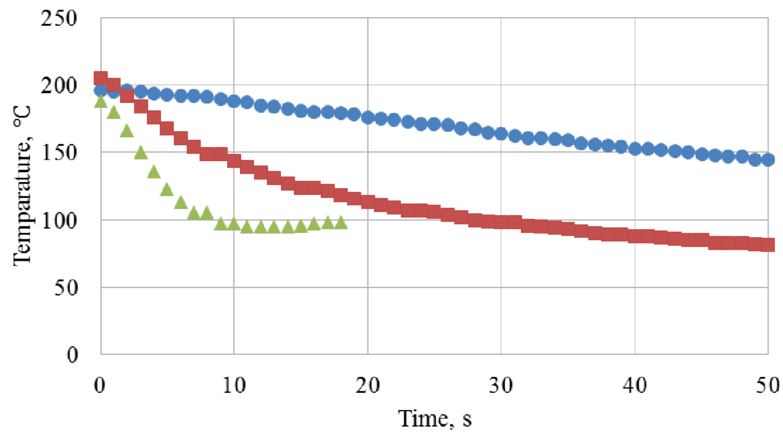

FIGURE 3. Thermograms of a flat copper plate interacting with gravity–moving dry sand with the values of mass flow: ● – 4.02 g/s; ■ – 45.5 g/s; ▲ – 427 g/s

It is seen from Fig. 3 that as the flow rate increases, the cooling rate increases as well. Since equation (1) includes parameter "a" – the thermal conductivity, it is logical to expect that the thermograms depend on this characteristic. It is not trivial to provide an identical instantaneous mass flow rate for various substances even under laboratory conditions, and in real conditions it is not always possible as well. Therefore, we should make comparisons based on relative indicators. If we consider the ratio of the instantaneous mass flow rate to the instantaneous rate of temperature change, then we get just such a criterion:

$$\frac{\left(\frac{dm}{d\tau}\right)}{\left(\frac{dT}{d\tau}\right)} = \frac{dm}{dT} = \kappa \tag{2}$$

This value characterizes the cooling of a solid surface by one degree of temperature by mass of bulk media streamlining it. Its dependence on the mass flow rate is important. The dependence is shown in Fig. 4.

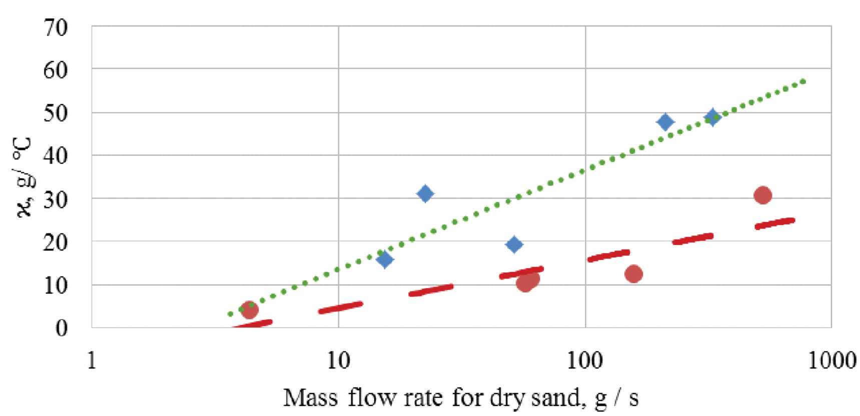

FIGURE 4. Dependence of parameter κ on the mass flow rate for dry sand taken when the plate is cooled by 30 ºC in the intervals: ♦ – 95…65 ºC; ● – 175…145 ºC

The data shown in Fig. 3 are taken from various spills, but the general patterns are traced quite accurately. As can be seen from Fig. 4, an increase in flow rate leads to an increase in κ as well. With an increase in the mass flow rate

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of the bulk medium, more granular media should be spilled to cool the sprinkled surface by one degree of temperature. A similar effect is described in [5], when, with an increase in the heating rate of bulk media, their ignition temperature increased. This result is completely in line with modern theoretical concepts, since it corresponds to the Second Law of Thermodynamics: irreversibility increases with an increase in the speed of the process. Thus, the efficiency decreases. Note that the parameter $\varkappa$ is the complete opposite to coefficient of efficiency for the cooling process surface i.e. with an increase in spilling rate, it should increase. It can also be seen from Fig. 3 that for the considered values, an increase in the mass flow rate of sand leads to a significant increase in the parameter κ by several times.

Below are the values of the parameter κ for spills of some of the substances we tested (Table 1).

TABLE 1. Heat transfer characteristics during spillage of various substances

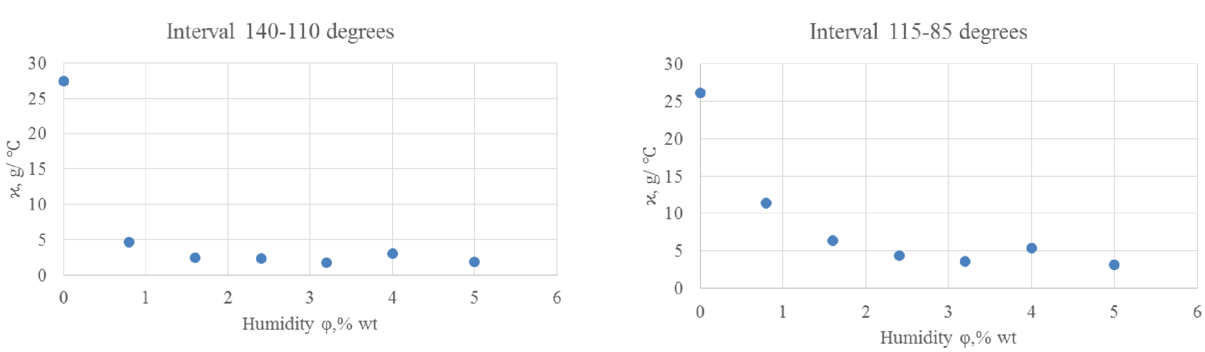
Substance	**κ, g/ °C**	**Cooling rate, °C/s**	**Thermal diffusivity a * 10^8, m²/s**
Water	3.35 … 4.12	14.1 … 17.6	13 … 14.3 [33]
Construction sand (GOST 8736–77)	2.45 … 79.8	0.45 … 11.7	26 … 32 [34]
Soda	3.95 … 13.8	2 … 7.6	2.3 …5.5 [34]
Semolina	8.03 … 38.3	0.86 … 2.26	6.48 [35]
Edible salt	9.45 … 25.2	1.57 … 5.73	30 … 110 [36]
Millet	12.2 … 64.3	0.34 … 1.8	6.0 … 11.5 [35]

For comparison, Table 1 also provides the plate cooling rates during spillage of bulk media and the thermal diffusivity coefficients of these media.

As can be seen from the above table, there is no clear correlation between the two considered characteristics – a and κ. Although from equation (1) this correlation is expected.

It can also be seen from Table 1 that the most effective cooling – both in speed and in relation (2) – occurs when the plate is exposed to water. In addition to the features of a substance, determined by its nature, the influence of humidity should be affected. And many studies confirm this [18, 19].

As noted above, food bulk media, when heated or humidified, are prone to irreversible changes in biological nature. Therefore, we tested the effect of moisture on the building sand. So, we are interested in the influence of humidity on the value of criterion (2). To do this, we dried the sand (GOST 8736–77) to the point where the bulk density is no longer affected by temperature. And this was taken for completely dry sand, as far as it can be taken in atmospheric conditions. Next, we added a certain amount of water to this dried sand to obtain the final granular sample of given moisture content. The literature describes the changes available for registration that occur in bulk media at sufficiently high humidity values: 6–8% [6], 10–20% [16], 40–70% [19]; therefore, it is of interest consideration of the area of lower values of humidity. The results we obtain for sand at the same values of the mass flow rate of 9 ... 13 g/s and the temperature range of cooling by 30 ºC are shown in Fig. 5.



a) b)

FIGURE 5. Dependence of the parameter characterizing the efficiency of cooling a flat copper plate with building sand on the moisture content of the sand: a – cooling from 175 to 145 ºC, b – from 115 to 85 ºC.

As can be seen from Fig. 5, the dependence of the parameter characterizing the cooling efficiency κ on the humidity of the environment in the considered range of values has a clearly defined behavior. Sand spillage

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corresponds to parameter κ at the level of 27 g/ºC. An increase in humidity φ by only 1% by mass leads to a significant decrease in κ (i.e., an increase in cooling efficiency). In this case, a further increase in humidity in the considered range does not lead to a change in the value of κ. For all points shown in Fig. 5, the sand mass flow value was maintained constant at 11 ± 2 g/s.

Sand is considered "dry" when the weight content of moisture does not exceed 5% [37]. With the mass humidity of 5%, sand holds its shape in the best possible way [38, 39]. Nevertheless, the two groups of points (Fig. 4) differ quite clearly: the data corresponding to cooling from 175 to 145 ºC are characterized by lower values of κ, i.e. more efficient cooling than the data corresponding to cooling from 95 to 65 ºC. Note that in both cases the temperature difference is the same – 30 ºC. However, as our results in Fig. 5 show, within the specified range – from 0 to 5% – there are two intervals: φ = 0 ... 1% and φ> 1%. Thus, the presence of even a small amount of moisture significantly affects the heat exchange between the cooled surface and the free–flowing medium. This is rather obvious: the evaporation of water is a very endothermic process [40] and should lead to intense cooling. The experiments in this work were carried out at atmospheric pressure, which corresponds to a boiling point of water of 100 ºC [41]. Thus, the difference between the slopes of the averaging lines in the results we obtained for "dry" sand (Fig. 4) can be explained by the effects associated with the phase transition of water.

In addition to the influence of humidity, one more factor should be mentioned. With initially equal values of the temperature of the granular medium, the temperature difference is present:

$$\Delta T = T_{tw} - T_{fm} \tag{3}$$

where T_{tw} – temperature of the cooled surface, ºC; T_{fm} – temperature of the free–flowing medium, ºC.

In fact, for the intervals 175 ... 145 ºC and 95 ... 65 ºC, the average temperature values differ by

$$(175+145)/2 - (95+65)/2 = 160 - 80 = 80\ ^{o}C,$$

accordingly, for this difference – 80 ºC – the values of the temperature for two shown cases are different.

Since the heat is removed from the heating surface is according to the Newton–Richmann Law ($q = \alpha\ \Delta T$, where q is the specific heat flux density, W/m^2, α is the heat transfer coefficient, W/(m^2 ºC), ΔT is the temperature head) [23] and it is proportional to the temperature head. With the increase in the temperature head the cooling efficiency increases as well (κ decreases). From a comparison of the graphs in Fig. 5 a) and b), it can be seen that for a larger value of the temperature head, the dependence of κ on humidity is more defined (steep).

Thus, the main heat transfer occurs in the wall layer. Layers that are more distant from the cooling surface (In our case – the upper layers) have their heat conduction defined by their thermal conductivity. Therefore, at a high flow rate, the layers farthest from the cooled surface do not have time to warm up and from the point of view of cooling the heating surface is obsolete. With the increase in flow rate, the efficiency of cooling the heating surface by a bulk medium decreases, which is clearly seen from the behavior of the dependences in Fig. 4. However, with an increase in the temperature difference, the temperature gradient increases. According to the Fourier Law ($q = -\lambda\ grad T$, where λ is the thermal conductivity coefficient, W/ (m ºC), *grad*T is the temperature gradient, ºC/m) [23], the transferred heat flux will also increase. It should be noted that in the case of a free–flowing medium, λ is the effective coefficient of thermal conductivity, which takes into account all the heat exchange processes.

Expanding the scope of experiments and clarifying the considered patterns is the subject of our further research.

Conclusion

Heat and mass transfer during the interaction of bulk media with solid surfaces takes place in various spheres of industrial activity: from the food industry to space exploration. To study heat and mass transfer processes under the mentioned conditions, we have created an experimental setup that makes it possible to simulate the gravity flow of bulk media around a heated flat plate. A series of experiments was carried out to study the cooling of a heated flat copper plate with a one–sided flow around it with various free–flowing media and water moving by gravity. Arrays of experimental data have been obtained.

1. It is proposed to evaluate the efficiency of cooling a solid surface by a bulk medium in relation to the mass flow rate of the medium to the rate of decrease in the temperature of the cooled surface. It is noted that this

relationship for the conditions considered in the work does not have a clear correlation with the thermal diffusivity.

2. It has been established that the value of this value increases logarithmically with an increase in the mass flow rate of the bulk medium.
3. It has been established that the values of this ratio depend on the temperature head between the cooled surface and the bulk medium: the higher the temperature difference, the smaller the amount of the flowing bulk medium leads to the cooling of the surface by 1 degree of temperature.
4. It has also been established that the dependence of the value of this ratio for construction sand changes sharply with a change in humidity from 0 to 1 mass percent and practically does not change in the range from 1 to 5 mass percent.

In the future, it is planned to improve the research methodology, modernize the installation and expand the research.

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Acaro-Entomological Studies of Pests Associated with Quarantined Plant Products

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Abstract. Plant quarantine is aimed at preventing the entry of pests that pose a threat to the biological and food security of our country. Activities in plant quarantine are based on the results of phytosanitary risk analysis to determine the compliance of a pest with the status of a quarantine object and diagnostic protocols. To develop the latter, it may be necessary to conduct special studies, including acaro-entomological. In this paper, studies have been conducted to develop methods for the diagnostics of insects and mites that pose a phytosanitary risk when importing plant products: strawberry budworm (Anthonomus signatus) — a quarantine object for the EAEU, and species that meet the criteria of quarantine objects for the territory of Russia: red palm mite (Raoiella indica), Californian elater (Limonius californicus), solanum fruit fly (Bactrocera latifrons). The species associated with exported grain products are also considered: flour mite (Acarus siro), indian meal moth (Plodia interpunctella), and mill moth (Ephestia kuehniella). A wide range of preparation and microscopy methods, including electronic scanning microscopy, was used to study the diagnostic signs of the pest species listed above. As a result of the conducted research, simplification of identification schemes was proposed to accelerate phytosanitary diagnostics; the applicability of diagnostic signs, including with confirmation by morphometry, was clarified to increase the reliability of diagnostic protocols; and a search for potentially significant diagnostic signs was carried out. In addition, improved methods of insect preparation have been proposed.

INTRODUCTION

The penetration and spread of quarantine insect and mite species may pose a threat to the biological and food security of the Russian Federation. Activities in plant quarantine are based on the results of phytosanitary risk analysis (PRA) to determine the compliance of a pest with the status of a quarantine object and diagnostic protocols. To develop the latter, it may be necessary to conduct special studies, including acaro-entomological.

In this paper, the issues of morphology and diagnostics of some regulated and proposed for regulation insect (Insecta) and mites (Acari) species, representing a phytosanitary risk for Russia when importing plant products and related to exported grain products, are considered.

Methods

Production of slide preparation. For Coleoptera, it was carried out using Canadian balsam; Diaspididae — based on Canada balsam with fuchsin staining; Acari — Hoyer's medium.

Preparation of Diptera and Lepidoptera genitals. The abdomen of male Diptera was separated, then placed in a 10% KOH solution for 2 hours, after which it was washed in distilled water, dissected, and stored in glycerin. For Lepidoptera, both classical "cold" and "hot" methods were used (aging in a 10% KOH solution) and combined — with the use of a micro thermostat.

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Microscopy and photographing were carried out on the basis of the All-Russian Centre of Plant Quarantine. Stereomicroscopes Zeiss Stemi 2000-C and Stereo Discovery.V12 were used to study insects. In some cases, Hitachi TM4000 Plus (SEM) electronic scanning microscope was used.

Zeiss Axio Imager A1 and Axio Imager 2 microscopes equipped with phase contrast were used to study slide preparations.

Photographing and morphometry were carried out using Zen 2.3 software, Zerene Stacker, and Adobe Photoshop CC.

The volume of the studied material is indicated by types of pests. The distribution of pests and their regulatory status is given according to EPPO and CABI databases [1, 2].

Results and discussion

Mites and insects that pose a phytosanitary risk when importing plant products

Red palm mite. *Raoiella indica* Hirst, 1924 (Arachnida: Acari: Tenuipalpidae) damages about 100 plant species, can be found in planting material, potted plants, and fruit products of tropical and subtropical crops. According to the results of PRA, the area at risk is the territory of the Southern Federal district of Russia. During the authors' own research in 2018-2020, *R. indica* has not been detected in Russia.

By using our own material (23 pcs. *R. indica*, Fig. 1) and illustrations, and photos of slide preparations [3, 4], a qualitative assessment of the lengths of dorsocentral setae (*c1*, *d1*, *e1*) among themselves and the ratio of the length of the first pair of dorsocentral setae (*c1*) to the distance between rows of *c1–d1* setae in *R. indica* and the remaining 20 species of the genus was carried out.

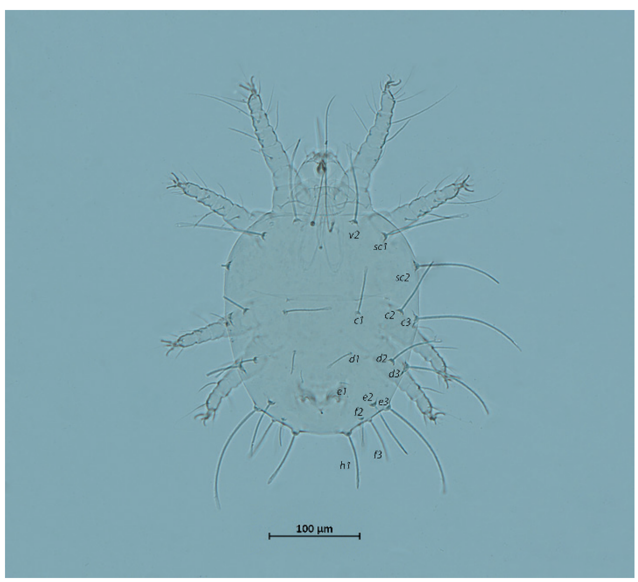

FIGURE 1. Female *Raoiella indica*, dorsally. The setae of the right half of the body are indicated by indexes, explanations in the text (photo by I. Kamayev)

R. indica is characterized by an expressed sequential decrease in the lengths of dorsocentral setae *c1>d1>e1*, while the length of *c1* exceeds the distance between the rows of setae *c1–d1*. In females of *R. indica*, the setae *h1* and *h2* differed in length slightly. The listed signs can be used in the development of a simplified identification scheme for the diagnostic protocol of the red palm mite.

Strawberry budworm. *Anthonomus signatus* Say, 1831 (Insecta: Coleoptera: Curculionidae) is widespread in Canada and the USA and considered a dangerous pest of berry crops, included in the list of quarantine organisms absent on the territory of the EAEU.

The genus *Anthonomus* includes 558 species from almost all parts of the world [5, 6]. Due to the lack of keys that unite species of this genus from different geographical regions in the literature, reliable identification of the

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strawberry budworm is difficult. At the same time, existing keys use a limited set of features for dry collectible items (for example, [7, 8]).

In this study, a search for new, previously unexplored morphological signs of *Anthonomus signatus* and two close species with similar food specialization: *A. rubi* and *A. bisignifer* was carried out.

The study of mouthparts [9] and apical tergites and abdominal sternites of 10 *Anthonomus* species showed no significant differences in the structure of these body parts. However, when studying slide preparations of the forelegs, signs that could potentially be used for diagnostic purposes were identified (see Fig. 2).

FIGURE 2. The structure of the forelegs of three species of *Anthonomus* genus (photo by S. Kurbatova)

Comparison of the forelegs of 3 *Anthonomus* species showed significant differences in the proportions of their 1st joint. In *A. rubi*, the base of this joint is more than 2.5 times narrower than the apex in its widest part and 8.5 times shorter than the total length of the joint. In *A. signatus*, it is 2 times narrower than the vertex and 6 times shorter than the total length, and in *A. bisignifer*, it is less than 2 times narrower than the vertex and less than 4.5 times shorter than the total length. The length of the 2nd joint in *rubi* is 1.35 times bigger than the maximum width, in *signatus* — 1.25 times, and in *bisignifer* — only 1.15 times bigger. The shape of the blades of the 3rd joint and the claws of the apex joint of the legs also differs significantly in the compared species. The same applies to the shape of the front shins.

Thus, these signs of the forelegs have undoubted diagnostic significance and can be used in the development of keys for the identification of *Anthonomus signatus*.

Californian elater. *Limonius californicus* (Mannerheim, 1843) (Insecta: Coleoptera: Elateridae) is a North American especially dangerous pest of potatoes, beets, wheat, corn, other crops.

Representatives of *Limonius* genus are common in the Old and New World [10], which makes it difficult to diagnose *L. californicus* in case of its invasion. In our study, we studied the typical material (2 syntypes) of Californian elater and developed keys for its differentiation from Nearctic and Palearctic species of *Limonius* genus.

Solanum fruit fly. *Bactrocera (Bactrocera) latifrons* (Hendel, 1915) (Insecta: Diptera: Tephritidae) is a pest of solanaceous crops, as well as some Cucurbitaceae, common in Asia, Africa, and Hawaii (USA).

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The exact diagnostics of species is often based on the structure of the genitals of males, which are not fully investigated in *Bactrocera* genus. The genitals of males (Fig. 3) of *B. latifrons* (3 pcs, Vietnam) and two quarantine objects of the EAEU were studied: *B. dorsalis* (Hendel, 1912) (5 ex., Thailand) and *B. cucurbitae* (Coquillett, 1899) (2 ex., Vietnam). The terminology is given according to [11].

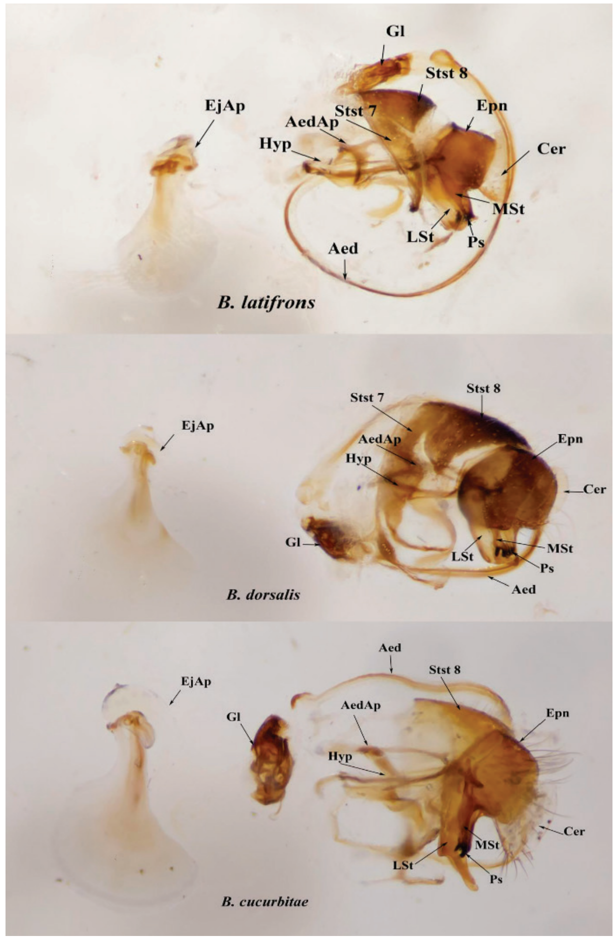

FIGURE 3. Genitals of males of *Bactrocera latifrons*, *B. dorsalis, B. cucurbitae* (photo by M. Arapova). Stst 8 – syntergosternite 8, Stst 7 – syntergosternite 7, Epn – epandrium, LSt – lateral surstyles, MSt – medial surstyles, Ps – prencissets, Cer – cerci (proctiger), AedAp – apodema of the aedeagus, Hyp – hypandrium, Aed – aedeagus (phallus), Gl – glans, EjAp – apodema of the ejaculator

Bactrocera genus was divided into subgenera *Bactrocera* and *Zeugodacus* on the basis of the structural signs of the surstyle — a paired structure that articulates with the epandrium, the ninth tergite of the male abdomen [10]. In *B. latifrons* and *B. dorsalis*, the posterior lobes of the lateral surstyles are short, which is typical for *Bactrocera* subgenus, and in *B. cucurbitae* they are long, as in other species of *Zeugodacus* subgenus.

In all three species, medial surstyles bear tooth-like bristles — prencisets. The proctiger of *B. latifrons* is membranous, smaller in size than the epandrium, bears light brown bristles, unlike the proctiger of *B. cucurbitae*, covered with dark brown bristles. The glans is sclerotized, with a well-developed acrophallus, narrow and elongated. The structure of the surstyles, proctiger, and glans is an important diagnostic sign.

Mites and insects associated with exported grain products

Flour mite. *Acarus siro* L (Acari: Acaridae) is regulated in grain products by Mexico, Brazil, and Nigeria. Three species are considered as part of *Acarus siro* complex: *A. siro* L, *A. farris* Oudemans 1905, and *A. immobilis*

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Griffiths, 1964 [12, 13]. The exact identification of these species is important for the establishment of pest free places and production sites.

One of the differentiating signs of the species of *A. siro* complex is the form of basal solenidium on the second pair of legs, Fig. 4 [13]. In total, 35 solenidia were studied in 19 individuals of *A. siro* from one population: apical expansion was implicit or not expressed at all in 40% of the studied cases. At the next stage, morphometry of the basal solenidium was performed on legs II (mean± standard deviation, measurement interval in parentheses), in µm: thickness of the distal extension – 4.13±0.45 (3.29-5.23); width of the tapering part – 3.50±0.42 (2.68-4.43); the width of the solanidium is approximately at the level of half of its length – 4.29±0.53 (2.98-5.24). Thus, the use of morphometry allows us to confirm the state of the diagnostic sign in *Acarus siro*.

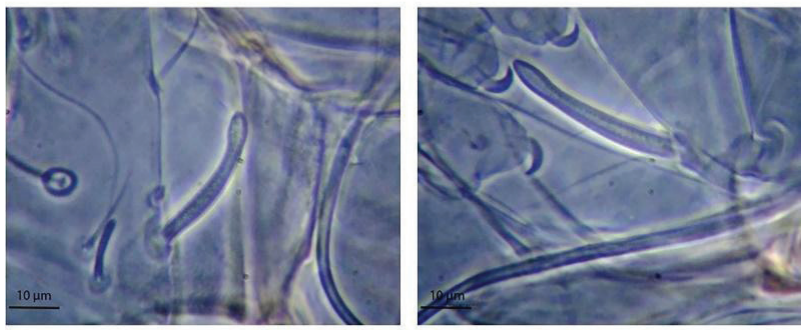

FIGURE 4. Apical extension of the basal solenidium on the tarsi of the first (*left*) and second (*right*) pairs of *Acarus siro* legs (photo by I. Kamayev)

Moths. Indian meal *Plodia interpunctella* (Hübner, 1813) and mill moths *Ephestia kuehniella* Zeller, 1879 (Insecta: Lepidoptera: Pyralidae) are regulated as quarantine in Egypt, *E. kuehniella* is also a quarantine object for Lebanon.

Despite the fairly well-studied biology of these species [15], some aspects of their morphology used in identification need to be clarified. Thus, on a large comparative material, it was shown that an important diagnostic feature – the common shield of the D1 and SD1 setae on A9 in *P. interpunctella* larvae, is completely invisible on fixed larvae. It was found that another diagnostic sign, the SD2 setae on A8, is clearly visible only on a scanning electron microscope (Fig. 5, inset). The data obtained will be used in the preparation of chaetotaxy schemes of the studied species and for the development of diagnostic protocols.

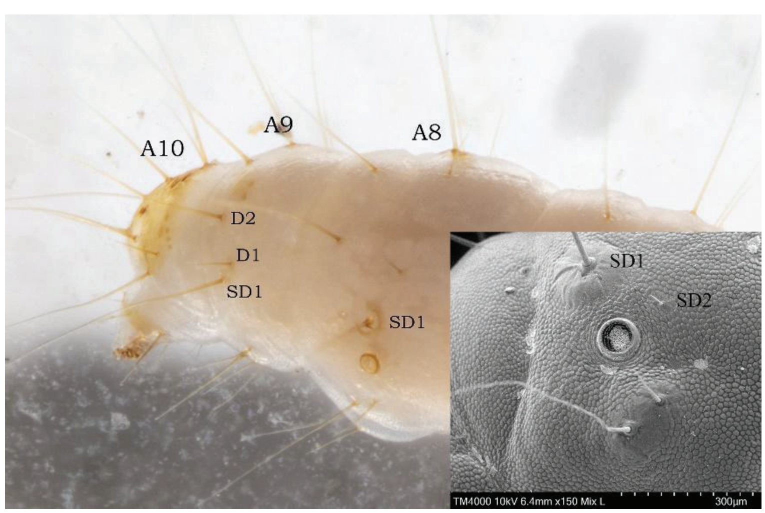

FIGURE 5. Diagnostic signs of *P. interpunctella* larva: caudal end of the body (A8-A10). The inset shows a scan (SEM) of a fragment of the eighth abdominal segment (A8), where SD2 is located in front of the stigma at a distance approximately equal to its diameter (photo by J. Lovtsova)

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It has been shown that the classical methods of preparing the genitals of males of *P. interpunctella* and *E. kuehniella* ("cold" and "hot") have a number of disadvantages when used in phytosanitary laboratories. As a result of the work carried out, a new method of preparation of the male genitalia preparation using BIS-H 209-01 microthermostat was recommended. It is shown that the optimal preparation time at a temperature of 80 °C is 30-50 minutes. Such duration of exposure makes all structures clearly visible, sclerites remain soft and probability of their deformation during preparation is minimal.

Conclusion

The conducted research in the field of diagnostics of insect and mite species regulated and proposed for phytosanitary regulation allows to:

1. use new diagnostic signs.
2. simplify identification schemes to speed up routine phytosanitary examination.
3. clarify the applicability of diagnostic signs and their confirmation by morphometry.

In addition, improved methods of insect preparation aimed at speeding up the diagnostic procedure are proposed.

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Phytosanitary Status, Diagnostic Methods for ToBRFV and Other Viruses of Tobamovirus Genus

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Abstract. Tobamoviruses like Tomato brown rugose fruit virus (ToBRFV), Tomato mosaic virus (ToMV), Tobacco mosaic virus (TMV), Pepper mild mottle virus (PMMoV), and Tobacco mild green mosaic virus (TMGMV) are dangerous pathogens of vegetable crops the family Solanaceae of the outdoor and indoor growing. Tomato and pepper yield losses as a result of infection with these pathogens can reach 100%. Early detection and eradication of outbreaks of these viruses are essential to prevent their rapid spread. ToBRFV is a dangerous contagious pathogen affecting tomato and pepper. It has a high rate of spread, is easily transmitted by contact and mechanically, retains infectivity for a long time on inert surfaces, and over the past few years, the virus has been detected in many countries on almost all continents, where it has caused very serious damage to vegetable growing. These features of ToBRFV necessitated its inclusion in the EPPO Alert List of Pests and the creation of international research groups to develop methods for its detection and identification. As a result of testing primers for simultaneous detection of the tobamovirus complex, one pair of primers was selected and conditions for PCR were optimized, allowing to detect all available isolates of Tobamovirus genus. Some available molecular diagnostic methods have been tested, which have shown their sufficient effectiveness for the detection and identification of pathogens. At the same time, the conducted studies have shown that it is necessary to continue improving the methods of detection and identification of tobamoviruses to improve the quality of laboratory tests.

INTRODUCTION

Currently, in the Russian Federation, indoor vegetable production is one of the leading areas of agriculture. Tomato is the most important vegetable crop, and its production in Russia is growing every year. According to experts, the gross crop of tomatoes in 2020 in the territory of the Russian Federation amounted to 560 TMT.

Tomato plants are hosts of more than 200 pests and diseases. Currently, pathogens of viral diseases are an important limiting factor for many branches of crop production, including vegetable growing, causing significant economic losses. In recent years, in European countries, the production of tomatoes and other solanaceous vegetable crops of the outdoor and indoor growing has suffered from serious losses caused by such viruses as: Tomato spotted wilt virus (TSWV), Pepino mosaic virus (PepMV), Tomato mosaic virus (ToMV), Tomato chlorosis virus (ToCV), Tomato infectious chlorosis virus (TICV), Tomato leaf curl New Delhi virus (ToLCNDV), Tomato yellow leaf curl Sardinia virus (TYLCSV) [1, 2]. However, the most acute problem for tomato production worldwide was Tomato brown rugose fruit virus (ToBRFV) and other tobamoviruses, of which TMGMV, TMV, ToMV, and ToMMV are the most economically important [3].

ToBRFV was first isolated from tomato plants grown in greenhouses in Jordan in 2015. The virus belongs to Tobamovirus genus, many members of which are widespread on the territory of the Russian Federation.

By having many pathways, including imported quarantined products, and a high rate of spread, ToBRFV has been detected in Europe, Asia, and America over the past two years, where it caused serious damage to tomato production. ToBRFV is highly pathogenic and retains viability for a long time not only in seeds, plants, and their parts but also on inert surfaces. An earlier analysis of phytosanitary risk showed that ToBRFV meets the criteria of a

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quarantine organism for the Russian Federation, can enter the country, acclimatize on the territory, and cause significant economic damage [1].

Due to the very rapid spread of ToBRFV in many countries, on July 7, 2021, this virus was included in the List of Quarantine Pests of the EAEU.

In the period 2018-2020, ToBRFV has been detected in many countries exporting tomato fruits and seeds to the Russian Federation. Taking into account the high ToBRFV pathogenicity and the possibility of its pathways on the territory of Russia, it is necessary to use highly sensitive and specific methods of its diagnostics.

A typical feature of the epidemiology of tobamoviruses is their ability to infect plants together with other viruses, and, therefore, an important element of the diagnostics of tobamoviruses is the differentiation of members of this genus.

Materials and Methods

The research was carried out based on the scientific department of the All-Russian Plant Quarantine Center in 2020-21. The main objects of research were viruses of Tobamovirus genus infecting Solanaceous vegetable crops: ToBRFV, ToMV, TMV, PMMoV, and TMGMV. Experiments were carried out with reference isolates of tobamoviruses from the DSMZ collection (Germany) and positive controls by Adgen (the UK) and Loewe (Germany).

For the isolation of nucleic acids, a set of “Proba-NK” (AgroDiagnostica, Russia) was used, for the reverse transcription (RT) — reagent kits, according to the manufacturer instructions: MMLV RT Kit (Evrogen, Russia); reagent kit for RT (AgroDiagnostica, Russia). Reagents Multiplex PCR Kit (Qiagen, USA); Screen Mix-HS (Evrogen, Russia); 5x Mix^{DD}Mix-2025 (Dialat, Russia) were used for PCR with primers ToMV-F/ToMV-R [4], ToMV-F1/ToMV-R1 [5], ToMV-F3/ToMV-R3 and TMV-F3/TMV-R3 [6], ToMV-FM/ToMV-RM and TMV-FM/TMV-RM [7], TMV-F2/TMV-R2 [8], TMV-MP-F/TMV-MP-R [9], TMV-spec/ToMV-spec [10], TMGMV-spec/Tob-Uni1 R/TMGMV CPTMF-F/TMGMV CPTMG-R [11] and the primers developed by the authors of the article — ToMV-P2F/ToMV-P1R and TMV-8PF-1/TMV-3PR.

To test the effect of different matrices on the reaction, 10 g of healthy tomato fruits of nine different varieties were used. Isolation was carried out in three-fold repetition for each sample.

One-step real-time PCR with reverse transcription (RT-qPCR) was performed with ToBRFV RNAs isolated from samples using a 2.5x Reaction mixture for qPCR (Syntol, Russia) with the addition of MMLV reverse transcriptase (Syntol, Russia), following the manufacturer's instructions. In the first case, primers ToBRFV-qs1, ToBRFV-qas2, and ToBRFV-p [12] were used for amplification.

In the second case, one-step RT-qPCR was also carried out using a “2.5x Reaction mixture for PCR-RT” with the addition of MMLV (Syntol, Russia), RT-qPCR was carried out with primers and a probe CaTa28 Fw, CaTa28 Rv, CaTa28 Pr [13].

The isolated RNA samples were also subjected to conventional RT-PCR in one test tube using a “2.5x Reaction Mixture for PCR-RT” with the addition of MMLV (Syntol, Russia), with ToBRFV-F/ToBRFV-R primers [14], to the area of the small subunit of replicase of TBRFV-Ps isolate. The expected amplification product is 560 bp.

In the second case, when setting up a one-stage conventional RT-PCR, a “2.5x Reaction mixture for qPCR” was used with the addition of MMLV (Syntol, Russia) and primers ToBRFV-FMX and ToBRFV-RMX [15], developed for the site of the RNA-dependent RNA polymerase of ToBRFV-IL isolate gene. The expected amplification product is 475 bp.

Amplification results were recorded after electrophoresis in 1.5% agarose gel. Were used molecular weight markers GeneRuler™ 100+ bps and Fast Ruler™ (Fermentas, Thermo Fisher Scientific, USA).

Results

The following results were obtained using one-step RT-qPCR with primers ToBRFV-qs1, ToBRFV-qas2, and probe ToBRFV-p, for samples of different initial masses.

Thus, when performing a one-step RT-qPCR with primers and a ToBRFV-qs1/ToBRFV-qas2/ToBRFV-p probe, a positive signal was obtained for all samples at low threshold cycles. It should be noted that when using the initial mass of 50µg homogenizate in the sample preparation process, the average signal Cq ranged from 22.3 to 27.8, and with an initial mass of 100µg, the average signal Cq ranged from 17.6 to 20.3.

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Thus, initial sample mass affects the reaction, when using a smaller mass, the threshold cycles are lower, which, with low contamination of the sample, can give false-negative results.

When setting up a one-stage RT-qPCR with primers and CaTa28 Fw, CaTa28 Rv, CaTa28 Pr probe, when using the initial mass of 50 µg homogenizate in the sample preparation process, two positive samples out of nine were detected. For all samples with an initial mass of homogenizate, a positive signal was obtained at low threshold cycles during sample preparation of 100 µg, the average Cp value was from 15.5 to 21.0.

Thus, when using primers and probe CaTa28 Fw, CaTa28 Rv, CaTa28 Pr in research we have a possibility of obtaining false-negative results when using a small subsample or a sample with low titer of virus.

When studying the identification of samples of different initial mass, using one-stage conventional RT-PCR with pairs of ToBRFV-F/ToBRFV-R and ToBRFV-FMX/ToBRFV-RMX primers, it was found that the studied pairs of primers amplify specific products of the expected value. No amplification products were found in negative control samples. It should be noted that when using a pair of ToBRFV-FMX/ToBRFV-RMX primers, clearer amplification products were obtained on electrophoregram.

Duplex PCR studies have been performed for simultaneous detection of TMV and ToMV using a combination of TMV-F3/TMV-R3 and ToMV-F3/ToMV-R3 primers [6]. After testing several PCR reagent kits, the Multiplex PCR kit (Qiagen, USA) was found to be the best option for PCR. This test was successfully tested to detect TMV and ToMV on tomatoes in several vegetable farms of the Russian Federation.

The multiplex test for simultaneous detection of TMV, ToMV, and ToBRFV was tested. The primers TMV FM/ TMV RM, ToMV FM/ ToMV RM and ToBRFV FM/ ToBRFV RM [7] were found to be highly species-specific, reacting only with target isolates. The optimal option of this test was determined using Multiplex PCR Kit (Qiagen, USA) and the reverse transcription kit (AgroDiagnostica, Russia).

Experiments with ToMV-F3/ToMV-R3 primers (Kumar et al., 2011), ToMV-spec/Tob-Uni1 R (Alishiri et al., 2013) and ToMV-2PF/ ToMV-1PR (VNIIKR) for ToMV revealed that the ToMV-F3/ToMV-R3 primers were not highly species-specific, which limits their use in confirmatory tests for ToMV. For primers ToMV-spec/Tob-Uni1 R and developed our primers ToMV-2PF / ToMV-1PR found high specificity to ToMV, which allows us to recommend these primers for the confirmatory test for the presence of ToMV.

To develop a confirmatory test for TMV presence, six pairs of primers complementary to different parts of the TMV genome were tested. It was found that primers TMV-F2/TMV-R2 [8], TMV 8PF-1/TMV 3PR (NMOVB VNIIKR), TMV-MPF/TMV-MPR [9], and TMV FM/TMV RM [7], which did not react with isolates of non-target tobamoviruses, are characterized by the highest specificity to the target object (TMV).

For TMGMM diagnosis, the use of TMGMV CPTMF-F/TMGMV CPTMG-R primers has been established [11] for highly specific detection of TMGMV.

Discussion

The conducted studies demonstrate that the use of all primers and probes for ToBRFV studied in this work when using extracts from samples in the volume of 100 µl, makes it possible to find and identify ToBRFV in all samples.

As can be seen from the obtained results, it is preferable to use the primers ToBRFV-qs1, ToBRFV-qas2, and probe ToBRFV-p [12] for RT-qPCR, primers ToBRFV-FMX, and ToBRFV-RMX [15] for RT-PCR, since PCR sensitivity when used is higher and the virus was detected in samples with a low concentration of the target object.

Primers have been identified that can reliably detect the presence of TMV, ToMV and TMGMV in plant samples.

Conclusion

The conducted studies have shown that it is necessary to continue improving methods for detecting and identification of Tomato brown rugose fruit virus ToBRFV.

A comparison of primer systems for virus diagnostics made it possible to determine a specific identification method: thus, the use of one-step RT-qPCR by primers ToBRFV-qs1, ToBRFV-qas2, and probe ToBRFV-p, positive result was obtained for all known positive samples regardless of the initial sample mass. Similar results were obtained using a one-step conventional RT-PCR with the primer pairs ToBRFV-F / ToBRFV-R and ToBRFV-FMX / ToBRFV-RMX.

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A duplex test for the simultaneous detection of TMV and ToMV as well as a multiplex test for the simultaneous detection of TMV, ToBRFV and ToMV were performed. The experiments performed using multiplex PCR indicate the undoubted prospects of the multiplex test according to the method of Yan et al (2020).

Six, three and two pairs of species-specific primers were tested for objective diagnosis of TMV, ToMV and TMGMV respectively. Highly specific primers that react only with isolates of target objects have been identified. These include primers TMV 8PF-1/TMV 3PR and ToMV-2PF/ToMV-1PR, developed at the All-Russian Plant Quarantine Center.

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Estimating the Distribution of Weed Plant Species and Contamination of Grain during Export

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Abstract. When exporting plant products, it is required to comply with the phytosanitary requirements set by the importing country. This fully applies to deliveries of Russian grain abroad, the volumes of which have increased dramatically in recent years. The authors analyzed the quarantine lists and requirements of 53 countries for the plants of the families Papaveraceae, Asteraceae, Polygonaceae, and Caryophyllaceae. The contamination of grain samples received by quarantine laboratories in 2019-2020 was analyzed. The volume of grain exports for 2017-2020 was analyzed to understand the potential risks. Detailed studies have been conducted on Papaveraceae and Asteraceae. The authors found that out of 11 poppy weeds present in Russia, the seeds of *Glaucium corniculatum*, *Fumaria officinalis*, *Papaver rhoeas* are found in the production. With regard to the Asteraceae family, the genus *Centaurea*, often mentioned in phytorequirements, was analyzed and the species which fruits can clog grain were identified: *Centaurea diffusa* Lam., *C. stoebe* L. (syn. *Centaurea maculosa* Lam.), *C. solstitialis* L. The information was collected and processed using open databases [2, 10]. The maps of the distribution of weed species in Russia were made using the Global Mapper program. The information on the distribution of weeds on the territory of the Russian Federation can be used to identify free phytosanitary zones. The data obtained will be in demand in the field of phytosanitary.

INTRODUCTION

In recent years, in connection with the rapid growth of grain exports and Russia's emergence as a market leader, the issues of fulfilling the phytosanitary requirements of importing countries of these products have become quite relevant. Russian grain is supplied to 138 countries of the world [1]. Grain products must comply with the phytosanitary requirements of these states and be free from weeds and other types of pests that pose a threat to their biological diversity [2]. Among all harmful organisms, it is weeds that cause the greatest damage to agriculture [3-8].

According to the Information System developed at the All-Russian Center for Plant Quarantine (FGBU "VNIIKR"), about 60 species of plants of the Russian natural flora are included in the lists of pests from different countries of the world and are regulated when importing plant products [9]. However, the information regarding the distribution of these species in the territory of Russia, their participation in the composition of weeds and field vegetation, as well as their relationship with batches of grain products sent for export are not always unambiguous. It is required to constantly update and generalize these data, taking into account the ongoing changes in the management system of the agro-industrial complex and the introduction of integrated plant protection products. It is necessary to correlate the types of weeds listed in the phytorequirements of the countries regarding the possibility of

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contamination of Russian grain products by them. The studies of this kind are practically absent in the world phytosanitary practice, although they are of great importance in solving phytosanitary problems. The purpose of the study is to analyze the phytosanitary requirements of countries importing Russian grain, update information on the distribution of weeds in the Russian Federation, and identify the relationship of these weeds with product batches.

Materials and Methods

Phytosanitary requirements were obtained from public sites [2, 10]. The occurrence of weed seeds in grain products was monitored according to the reports of the testing laboratories of the FGBU "VNIIKR".

The information on the distribution of weed plant species in Russia was obtained from open databases [11, 12]. Plant distribution maps were compiled in the GIS Global Mapper. The export volumes of grain products with the risk of contamination by species regulated by importing countries was assessed according to the website of the ASD "Access TSVT" [1].

Results

The phytosanitary requirements of 53 countries importing Russian grain were analyzed in order to identify weeds prohibited for import into these countries. Most of these plants are found in the crop growing zone on the territory of the Russian Federation, their seeds and fruits may appear in batches of finished products of wheat, barley, rye, oats, rice, buckwheat, etc. It appeared that a significant proportion of taxa from the total list of weeds belongs to plants from the families Papaveraceae, Asteraceae, Polygonaceae, and Caryophyllaceae.

Nine countries completely ban the presence of Papaveraceae seeds in the batches of grain. It was established that the genus Papaver in its entirety and certain species of *P. rhoeas, P. dubium, P. hybridum, P. somniferum, P. orientale, Glaucium corniculatum, Fumaria officinalis, F. vaillantii, F. bastardii, F. densiflora, F. muralis* are regulated when importing products (Table 1).

TABLE 1. Importing countries that regulate poppy and cornflowers in grain products.

Name in Latin	Country	Phytosanitary status of the taxon in the country	Distribution in Russia
Papaver L.	Arab Republic of Egypt	Quarantine object (QP)	Everywhere
Papaver L.	Lebanese Republic	Regulated non-quarantine pest	Everywhere
Papaver dubium L.	Democratic Socialist Republic of Sri Lanka	Quarantine object (QP)	Locally
Papaver hybridum Spenn.	Democratic Socialist Republic of Sri Lanka	Quarantine object (QP)	Locally
Papaver somniferum L.	Democratic Socialist Republic of Sri Lanka	Quarantine object (QP)	Everywhere in culture
Papaver rhoeas L.	Republic of Uganda	Quarantine object (QP)	Widely
	People's Republic of Bangladesh	Quarantine object (QP)	
Papaver monanthum Trau.	Republic of Armenia	Regulated non-quarantine pest	Absent
Glaucium corniculatum (L.) Curtis	Lebanese Republic	Regulated non-quarantine pest	Widely
Fumaria officinalis L.	Lebanese Republic	Regulated non-quarantine pest	Widely
	Republic of Uganda	Quarantine object (QP)	
	Republic of Peru	Quarantine object (QP)	

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	People's Republic of Bangladesh	Quarantine object (QP)	
Fumaria vaillantii Loisel.	Republic of Armenia	Regulated non-quarantine pest	Widely
	Mexico	Potential quarantine object	
Fumaria bastardii Boreau	Federative Republic of Brazil	Quarantine object (QP)	Absent
Fumaria densiflora DC.	Federative Republic of Brazil	Quarantine object (QP)	Locally
Fumaria muralis Sond. ex W.D.J. Koch	Federative Republic of Brazil	Quarantine object (QP)	Absent
Centaurea diffusa Lam.	India, China, Brazil	Quarantine object	Widely
Centaurea stoebe L.	Pakistan	Quarantine object	Widely
Centaurea solstitialis L.	Canada	Quarantine object	Locally
Centaurea melitensis L.	India	Quarantine object	Absent

Source: Compiled by the authors.

With regard to the group of plants of the family Asteraceae, in the requirements of countries, *Acroptilon repens* L. is most often found, which is regulated by nine countries (Egypt, Brazil, Chile, Israel, Jordan, Azerbaijan, Georgia, Moldova, and Ukraine), is included in the Unified List of Quarantine Objects of the EAEU. Moreover, there are some species of thistles in the quarantine lists of countries: *Carduus nutans* (Taiwan), *Carduus acanthoides* (Venezuela) and cornflowers (*Centaurea melitensis* L. – India; *Centaurea diffusa* Lam. – India, China, Brazil; *Centaurea stoebe* L. – Pakistan; *Centaurea solstitialis* – Canada. Closely related to cornflowers, *Carthamus lanatus* L. is regulated by the requirements of Mexico (Table 1).

Figures 1 and 2 show the original distribution maps of some of the above-mentioned species, compiled according to the GBIF and AgroAtlas data [11-12].

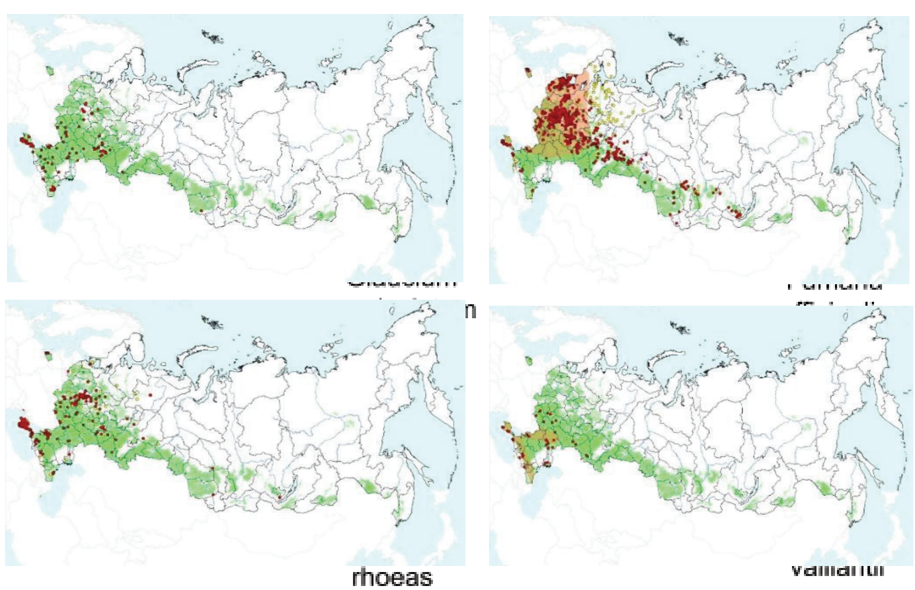

Figure 1. Papaveraceae species, widely distributed in Russia. *Source:* Compiled by the authors

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The analysis of the research data conducted by the testing laboratories of the Federal State Budgetary Institution "VNIIKR" in 2020 enabled to identify the main types of weeds. Thus, seeds of *Glaucium corniculatum, Fumaria officinalis, Papaver rhoeas* are regularly detected. The following are less commonly found: *Fumaria schleicheri, Glaucium flavum, Papaver setosum, Papaver nudicaule, Hypecoum erectum, Chelidonium majus, Corydalis sibirica*. *Papaver dubium* seeds are very rare. Among Asteraceae, achenes of cornflowers were most often found in Russian products: *Centaurea cyanus* L., *Centaurea depressa* L., *Centaurea diffusa* Lam.; much less often – *C. scabiosa*, *C. jacea*, and *C. phrigia* – were not detected at all.

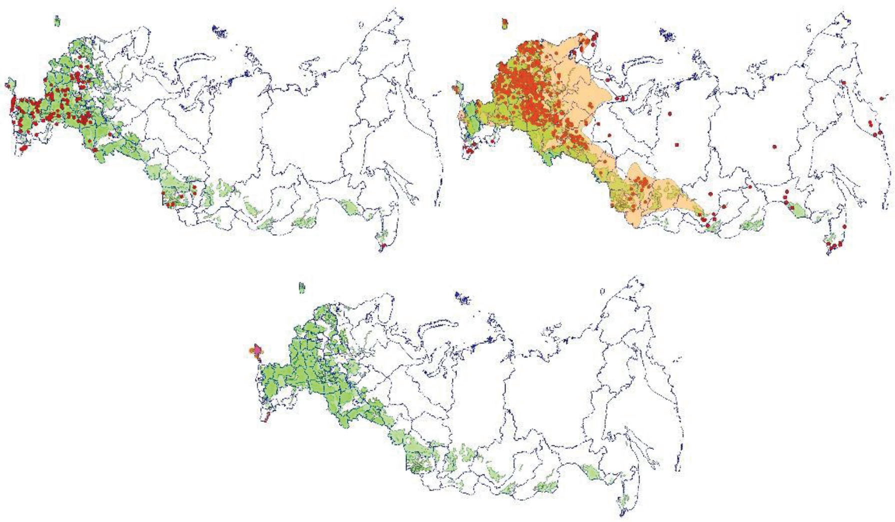

FIGURE 2. Species of the genus *Centaurea*, widely distributed in Russia. *Source*: Compiled by the authors

The analysis of the data of the customs statistics of the Russian Federation in the areas of export of grains and oilseeds enabled to establish that for the period 2018-2020, the total volume of products with the risk of contamination by poppy amounted to more than 36 million tons, and by cornflowers – 5.5 million tons (Table 2).

TABLE 2. The volume of export products with the risk of contamination by poppy and cornflowers in 2018-2020

Importing country	Volume of exports with the risk of contamination, thousand tons	
	poppy	cornflowers
Egypt	24010	-
Bangladesh	6440	-
Lebanon	2080	-
Mexico	1190	-
Armenia	1110	-
Sri Lanka	720	-
Uganda	580	-
Peru	180	-
Brazil	10	0.36
China	-	4263.19
Pakistan	-	1189.97
Canada	-	2.66
India	-	0.002
Total, million tons	36.31	5.46

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Total, million tons per year	12.10	1.82

Source: Compiled by the authors.

Discussion

When sending grain for export, the phytosanitary condition of the batches of products is established. The samples of regulated products are necessarily checked, as well as the production sites can be checked.

In the seeds of grain crops, 8 species of the Poppy family are found: 3 species of the genus *Papaver*, 2 species of the genus *Glaucium*, and 3 species of the genus *Fumaria* [13]. Poppy seeds quite regularly infest batches of wheat, barley, millet, rapeseed, sunflower, mustard, peas, chickpeas, soybeans, and coriander.

One of the most dangerous weeds of this family is *Papaver rhoeas* L., a segetal and ruderal weed that is resistant to herbicides. This species predominates in winter wheat, where it causes the greatest yield losses. The species is widely distributed in the territory of the Russian Federation from forest to desert zones. In the Far East, Siberia, and the Urals, this poppy is not found, thus these territories can be considered free zones. *Papaver dubium* L., which is locally distributed in the Black Sea region, Crimea, and the Caucasus, is very rarely found in products.

Other poppy weeds that are often found in grain products of Russian origin are *Glaucium corniculatum* (L.) Curtis and *Fumaria officinalis* L. *G. corniculatum* – an annual plant, an apophyte, is a segetal and less often ruderal weed, often found in the steppe and forest-steppe zone along rocky slopes. The species is widely distributed in the territory of the Russian Federation. In the Urals, Siberia, and the Far East, it is an adventive plant and is rare. *F. officinalis* L. is widely distributed in the zone of active crop production, and is also quite abundant in the seeds of spring crops, which allows to consider this species as segetal-ruderal weed. The phytosanitary requirements of Lebanon, Uganda, Peru, and Bangladesh prohibit the presence of its seeds in grain products. Other species of fumitories, present in the territory of the Russian Federation (*F. vaillantii* Loisel*., F. densiflora* DC.) were not detected in export grain products.

Weeds of the family Asteraceae are widely represented in the phytosanitary requirements of countries importing Russian products. The dominant part of this list belongs to the tribe Cynareae of the subfamily Carduoideae to the genera *Cirsium, Acroptilon, Centaurea, Carduus, Carthamus, Onopordum,* etc. These plants are quite widespread in the flora of Russia, often found in segetal and segetal-ruderal plant communities, litter the seeds of agricultural crops in the territory of the Russian Federation.

It is suggested to dwell in more detail on the largest genus of the family, *Centaurea* L. Cornflowers are widespread in the flora of Russia and occupy various natural phytocenoses in vast areas of the temperate zone of the Northern Hemisphere [13–15]. About 12 types of cornflowers are found in the central part of European Russia. About 10 types of cornflowers can grow in the fields, especially after plowing steppe areas.

Centaurea cyanus L. infests spring and winter crops, tilled crops, and perennial grasses. A taxonomically close species to it – *Centaurea depressa* M. Bieb. – is quite often noted in the composition of the segetal plant communities of the Crimea. The first species is an archaic weed that is historically associated with the seeds of grain crops and does not have natural habitats outside of agrocenoses. The frequency of occurrence of its fruits in products has noticeably decreased in recent years. Other types of cornflowers are ruderal or weedy ruderal plants that settle on pastures and fallow lands: *Centaurea diffusa* Lam., *C. stoebe* L. These species are introduced into North and South America, where they are aggressive weeds that cause significant economic damage [16]. Most of the sites they occupy in Russia are associated with secondary habitats (roadsides, garbage places, dumps, compacted soils), but from time to time they are noted near fields and as part of plant products. As weeds in the fields, perennial species are of lesser importance: *Centaurea jacea* L., *C. phrigia* L., *C. scabiosa* L. Achenes of *C. scabiosa* were found by quarantine laboratory staff in 22 cases, *C. jacea* – in 4 cases, and *C. phrigia* were not detected at all.

In the southern regions of Europe (Mediterranean, Crimea, Caucasus, Western and Asia Minor), mainly on irrigated lands and in weedy places, the following species are found – balsam cornflower C*entaurea balsamita* L., sun cornflower *C. solstitialis* L., prickly cornflower *C. calcitrapa* L., sheep cornflower *C. ovina*, splayed cornflower *C. squarrosa* L. [13]. Achenes of balsam cornflower were found in grain samples in 7 cases in 2019, and the achenes of sun cornflower – in 3 cases in 2020.

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Conclusion

Assessing the volume of export products with a risk of poppy contamination showed that the most significant weeds of Russian grain are the genus *Papaver* and *Papaver rhoeas* L., which are regulated by the phytosanitary requirements of Egypt, Lebanon, Bangladesh and Uganda. In products (wheat, barley, rapeseed), the seeds of *Papaver rhoeas* L. are detected more frequently than others.

Among the common cornflowers in Russia, only three species were identified that are regulated by the requirements of India, China, Brazil, and Pakistan (*Centaurea diffusa* Lam., *Centaurea stoebe* L., *Centaurea solstitialis* L.). According to the data of quarantine laboratories, cornflower achenes are occasionally found in grain samples, but, as a rule, in a single quantity.

All of the above-mentioned indicates the potential possibility of fulfilling the phytosanitary requirements of the importing countries of Russian grain in relation to weeds of the Poppy family and the genus Cornflower. Further study of the representation of these species in the grain-producing regions of the Russian Federation will allow to regionalize and identify the zones, which are free from these weeds. In areas of mass distribution of these weed species, it is required to strengthen the fight against them in the fields.

This information will be widely demanded by experts of quarantine laboratories and phytosanitary service.

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Protecting Forests from Pests as a Factor in the Stability of the Ecological System

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Abstract. The main objective of the NPPO is to prevent the introduction of harmful quarantine organisms. A brief review of forest pests included in the list of quarantine objects of the EAEU, including 46 species of insects, 1 species of mite, 1 species of nematode and 16 species of phytopathogenic fungi, is given. Most of the research is devoted to the pine wood nematode *Bursaphelenchus xylophilus*, as the most dangerous pest for coniferous plantations of the Russian Federation. Since 2010, the surveys have been conducted in 19 regions of the Russian Federation and about 6000 samples have been analyzed. The species *B. xylophilus* has not been found in the territory of the Russian Federation. The surveys of deciduous stands confirmed the absence of Asian (*Anoplophora glabripennis*) and Chinese (*A. chinensis*) longhorn beetles in the Russian Federation, which can be introduced with plants for planting from Europe or with wood packaging materials from China. The program for 2021-2023 has been developed on monitoring and improving measures to control the oak lace bug *Corythucha arcuata*, which is actively expanding its range in the Russian Federation. The outbreak of chestnut gall wasp *Dryocosmus kuriphilus* on 22,000 ha of chestnut was formed in the Krasnodar Krai as a result of an invasion of this pest from abroad. It is assumed that the release of *Torymus sinensis* parasitoids in the spring of 2021 will lead to the recovery of the sweet chestnut *Castanea sativa* in 4-6 years.

INTRODUCTION

An important factor in the ecological stability of the region is healthy forest plantations, including parks and tree windbreaks. Protecting forest stands from invasive pests is essential to maintaining healthy forest stands. Over the past 20 years, about 35 invasive species of forest pests have been detected in Russia. Some of them are quarantine species that, despite phytosanitary barriers, entered and established in the territory of the Russian Federation [1, 2].

The Unified List of Quarantine Organisms of the EAEU (Armenia, Belarus, Kazakhstan, Kyrgyzstan, and Russia) includes 237 species, and a quarter of them are associated with forest. Below one may find a brief analysis of the research conducted by the staff of the forest quarantine department of the FGBU "VNIIKR" on the most harmful quarantine forest pests.

Materials and methods

One of the main documents that determine the strategy for protecting forest plantations from pests-invaders is the official list of quarantine objects of the country, which is formed on the basis of pest risk analysis. The current Unified List of Quarantine Objects of the EAEU includes two sections: the species absent in the territory and the

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species of limited distribution. When an organism is included in this list, a list of forest products from which pests can be imported is formed, and then the country's phytosanitary requirements for the import of a particular plant product are developed.

Results and Discussion

The list of quarantine organisms of the EAEU includes 64 forest organisms: 46 species of insects, 1 species of mite, 1 species of nematode and 16 species of phytopathogenic fungi. Most of these organisms (75%) are of North American origin (Fig. 1).

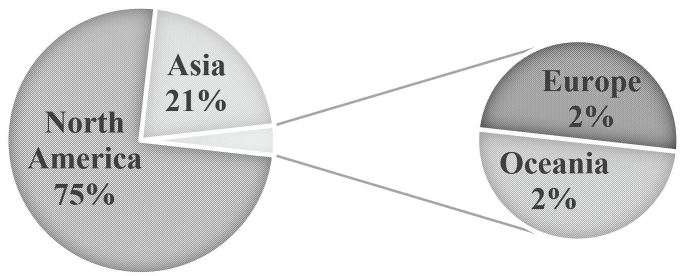

FIGURE 1. The number of forest organisms included in the list of quarantine pests of the EAEU and the regions of their origin

Nematodes. The pine wood nematode (PWN) *Bursaphelenchus xylophilus* is native to North America and is one of the most damaging conifer pathogens in the world. Native conifers in North America are resistant to this pathogen. With the introduction of PWN into Asia (Japan, China, and the Republic of Korea) at the beginning of the last century, the pathogen began to gradually spread, causing mass death of pine trees. In Europe, PWN was detected in 1999 in Portugal [3]. Nematodes in forest areas are spread by longhorn beetles *Monochamus* spp. In this regard, the North American longhorn beetles of this genus (8 species), as well as the Japanese longhorn beetle *M. alternatus*, are included in the A1 list of EAEU quarantine organisms. Taking into account the fact that PWN is widespread in the countries bordering the Russian Federation, the forest plantations in the country are annually monitored for the presence of this pathogen (Fig. 2).

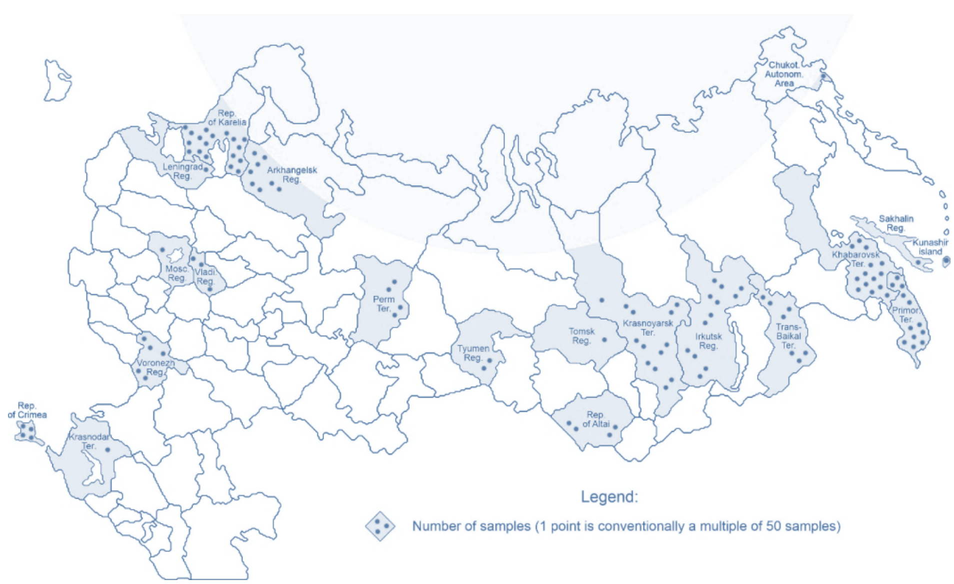

FIGURE 2. Regions of surveys of coniferous plantations for the presence of pine wood nematode in the Russian Federation territory

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The examinations of coniferous plantations in the territory of Russia for the presence of PWN were started by FGBU "VNIIKR" in 2010. From 2010 to 2020, 5966 wood samples were analyzed in eighteen regions of the country (Fig. 2). When identifying nematodes, morphological and molecular methods were used: classical PCR followed by sequencing, PCR in FLASH format or real-time PCR. The studies have shown the absence of PWN in the territory of the Russian Federation, but the closely related species *B. mucronatus* was often detected.

In response to calls to limit the use of methyl bromide worldwide, which depletes the Earth's ozone layer, the trials have been conducted to establish suitable gas concentration standards for the new fumigant ethandinitrile when treating roundwood against PWN [4].

Nematological surveys of deciduous plantations were conducted, where, when determining the causes of death of elms *Ulmus laevis, U. glabra* in parks in the Leningrad and Moscow regions, a new species for science *Bursaphelenchus ulmophilus* [5] was identified, which is a carrier of the pathogenic fungus *Ophiostoma novo-ulmi*.

Insects. Among the North American species quarantined for the Russian Federation, there is the bronze birch borer *Agrilus anxius*, which is so far distributed only in the USA and Canada. It has been established that the warty birch *Betula pendula*, widely growing in Russia, is a good host plant for this pest [6].

Other North American species of the genera *Acleris, Choristoneura, Malacosoma* can be introduced into the Russian Federation with plants for planting, but have not yet been found in other areas of the world, but bark beetles *Ips calligraphus. I. grandicollis* and *Dendroctonus valens* have recently been detected in China [7, 8]. At the same time, the death of pines from the bark beetle *D. valens* was noted [9]. It can be assumed that these bark beetles will soon appear in the Russian Far East territory.

The primary range of the bugs *Corythucha arcuata, Leptoglossus occidentalis, Corythucha ciliata* is North America. The oak lace bug *C. arcuata* was first detected in Europe, in Italy, in 2000 and has now spread widely on the continent [10, 11]. In 2016, the bug outbreaks were found in the Krasnodar Krai, Crimea, and Adygea. The pest intensively spreads, occupying its range associated with oak stands. Considering this, the oak lace bug and the seed bug *L. occidentalis* (outbreaks in the Krasnodar Krai and the city of Sevastopol) are recommended for transfer to the section of limitedly distributed quarantine organisms A2. A research program has been developed to improve measures to control oak lace bug for 2021-2023.

Longhorn beetles of the genus *Anoplophora* pose a great threat to forest plantations. The area of origin of the pests is China and neighboring countries, but the Asian longhorn beetle *A. glabripennis* has been introduced and is spreading in the USA, Canada, and a number of European countries [12]. The Chinese longhorn beetle *A. chinensis* is present in Italy [13]. These pests have not been detected in Russia, but their acclimatization is possible. The eradication of outbreak of these longhorn beetles is extremely difficult, and the only positive experience in the outbreak eradication was obtained only in Austria, where dogs were used to detection infested trees [14].

The chestnut gall wasp *Dryocosmus kuriphilus* is native to Asia (China). A pest outbreak with an area of 22,000 hectares was found in the Krasnodar Krai. Probably, the pest came from China to Europe, and then with plants for planting to the Russian Federation. The peculiarity of the situation is that chestnut plantations are located in an ecologically clean area where the use of pesticides is prohibited. In this regard, a biological control method was used, as in Europe [15] – the release of *Torymus sinensis* parasitoids to suppress the pest number in May 2021. It is expected that in 4-6 years this will lead to the improvement of chestnut plantations *Castanea sativa.*

The primary range of the ash borer *Agrilus planipennis* is Asia, including the Russian Far East. Not being a harmful species here, the borer introduced to North America and the European part of the Russian Federation began to intensively "destroy" ash trees [16]. Gradually, the pest spread widely throughout the European part of the Russian Federation. Similar problems with the ash trees death are observed in the United States, but Russia has its own characteristics. Following the development of a new territory by the borer, after 3-4 years, the dispersal of its parasitoids occurs, and the pest abundance decreases to a minimum [17].

Fungal pathogens. Another organism that kills ash trees is the causative agent of necrosis *Chalara fraxinea* (=*Hymenoscyphus fraxineus*). The species is fairly widespread in Europe, and there is information that it is present in the European part and the Far East of the Russian Federation [17, 18]. According to scientists [2], it was introduced from Asia with the plants for planting of the Manchurian ash *Fraxinus mandshurica*.

The causative agent of oak vascular mycosis *Ceratocystis fagacearum* (=*Bretziella fagacearum*) infects about 50 oak species in the United States, causing significant damage to forestry. *C. fagacearum* is absent from Europe and Russia, but according to the studies [19] European oak species, including English oak, are susceptible to this pathogen.

For pine plantations in Russia, the brown spotted needle blight of pine *Mycosphaerella dearnessii* (= *Lecanosticta acicola*) poses a significant danger. The primary range of the pathogen is North and Central America. The pathogen has spread to a number of European countries and East Asia, and may be present in Russia. The

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damage from the disease is expressed in a decrease in the annual growth of wood up to 75% per year. It can be introduced with plant for planting.

Pathway of harmful organisms. Of all the pathways, plants for planting pose the greatest phytosanitary risk, since they may contain almost the full range of quarantine pests. Even with careful inspection of plants by an inspector, it is almost impossible to detect organisms, especially at the initial stage of infection. Given this, most countries of the world prohibit the import of live plants from those areas of the world where quarantine organisms are common. For example, the import into the EU of a number of species of live plants from North America is prohibited due to the possible introduction of PWN, bark beetles, weevils, fungi, etc.

The same organisms can be introduced with unbarked wood (round wood, wood chips, and bark). Such products are subject to measures (thermal drying, chemical impregnation, fumigation, etc.) that guarantee the complete death of quarantine organisms.

Some organisms can move with transport. By laying eggs on the surface of vehicles (vessels, cars, containers), organisms can "move" over considerable distances in a short time. For example, the Asian subspecies of the gypsy moth *Lymantria dispar asiatica*, laying eggs on the deck superstructures of ships in the ports of the Far East, can successfully "get" to any port in the world which the ship enters.

It is possible to introduce pests with wooden packaging, which is used to fasten various products. Given the large trade between countries and the high pest risk, an international standard, ISPM 15, has been developed for wood packaging materials.

Conclusion

The key task of the NPPO is to develop regulations on applying quarantine phytosanitary measures to prevent the introduction, as well as to localize and eliminate quarantine pests. This is especially important for invasive forest organisms that have recently entered the country. Having analyzed publications on forest quarantine organisms, which are of limited distribution in the Russian Federation, it should be noted that very few studies have been devoted to the development of measures to control them. The development of effective measures to control dangerous forest pests is the main task in the field of protecting forest.

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Ways of Possible Introduction of Quarantine Forest Organisms with Wood Packaging Materials

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Abstract. One of the sources of introduction of harmful organisms from one country to another can be wood packaging materials (WPM). Despite the application of the international standard ISPM No. 15, NPPO staff in different countries periodically detect quarantine organisms in them. The analysis performed from insect and nematode registrations showed that the organisms were most often found in WPM that entered the EU with consignments from the Republic of Belarus (57, 92 and 73 detections, respectively, in 2017, 2018, 2019) and China (96, 52 and 56), then India (44, 36 and 23) and Ukraine (9, 29 and 7). In the WPM from Russia, living organisms were found 6 times in 2017 and 11 times in 2018, 2019, however, the identified objects were not quarantined. Among the quarantine organisms most frequently detected in the WPM are the pine wood nematode *Bursaphelenchus xylophilus,* the Asian longhorn beetle *Anoplophora glabripennis,* and the Chinese longhorn beetle *A. chinensis*. Nematode *B. xylophilus* in 2017-2020 was found 2 times in WPM with cargoes from China and once – from the USA (2019). The Asian longhorn beetle was identified 4 times in 2018-2020 in WPM with cargo from China.

INTRODUCTION

The risk of introduction with the products of various harmful organisms is growing every year due to the increase in trade between countries. Despite phytosanitary measures introduced by the national plant protection organization (NPPO), instances of pest infestations are periodically recorded by quarantine officers in many countries of the world.

The introduction of harmful organisms can be carried out with plants for planting, with lumber, wood chips, bark, etc. Some organisms are able to move considerable distances independently by flight or with a wind flow. Such a group of insects as, for example, bugs (*Corythucha arcuata, C. ciliata, Leptoglossus occidentalis),* butterflies *(*Malacosoma spp.*, Lymantria* spp.) often use vehicles (cars, ships, wagons) as a way to resettle their population, and for a few days they can be moved hundreds or thousands of kilometers. Another route of invasion by organisms is through wood packaging materials (WPM), which are used to secure cargo during transportation.

A country's NPPO may ban the importation of specific plant products based on the risk of infestation (e.g. EU embargo on untreated wood from North America in the late 1980s due to possible introduction of the pine wood nematode), but full trade cannot be stopped between countries. The information presented below includes an analysis of data on the facts of the detection and spread of harmful hazardous organisms transported with WPM, and an assessment of their possible invasion into the territory of the Russian Federation.

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Materials and Methods

Wood packaging materials (including pallets) exported with goods to the EU are subject to mandatory inspection by NPPO staff. All discrepancies and facts of detection of living organisms are recorded, entered into a single database INTERNET EUROPHYT (2021) and displayed in EPPO statistical reports [1]. These resources made it possible to analyze data on detections of various organisms in the WPM for 2017-2020. In conditions of intensive trade between countries, such information is updated annually [2-4].

Results and Discussion

Wood packaging materials are wood or wood products used to secure, protect or transport commodities. They should comply with the requirements of the International Standard for Phytosanitary Measures ISPM No. 15 [5].

According to INTERNET EUROPHYT [6], there are three types of wooden packaging and fasteners in which living organisms have been recorded: wood pallet, wood crate, and dunnage.

Fig. 1 shows data on the number of detections of living organisms in various types of WPM. For 2017-2019, 679 detections were registered. Most living organisms were found in pallets that are transported along with cargo – 61% of detections. Wood, which is used to secure cargo, accounts for 27% of detections. In a wooden crate designed to protect cargo from deformation and damage – 79 cases of detection of living organisms (12%). The presence of numerous registrations of organisms in pallets is explained by the fact that organisms survive better in wood of a larger mass. Often pallets are independent cargo and are declared as a separate item, but in this case they are not WPM. The species of the following taxa are most often found in the WPM: Anobiidae, Bostrichidae, Buprestidae, Cerambycidae, Curculionidae, Isoptera, Lyctidae, Lyctidae, Oedemeridae, Scolytidae, Siricidae, Nematoda (Bursaphelenchus, Aphelenchoides).

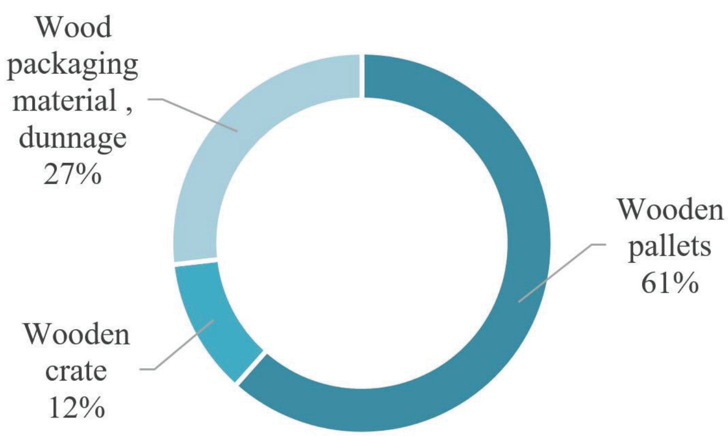

FIGURE 1. Number of detections (in %) of the total number of detected pests (insects and nematodes) in wood packaging materials delivered with consignments to the EU countries in 2017-2019 (according to EUROPHYT)

Quarantine organisms are sometimes found among the insects and pathogens detected in WPM. It is with WPM that in recent decades such dangerous organisms have been brought to the territory of different countries, as the pine wood nematode *B. xylophilus* and its vectors, the longhorn beetles *Monochamus* spp. [7], Asian longhorn beetle *Anoplophora glabripennis* (to Europe and North America), Chinese longhorn beetle *A. chinensis* – to Italy [8].

Imported plant products are required to be inspected by NPPO personnel, and pallets, crates, and lashings are usually not inspected due to the complexity of the controls. In order to prevent the possible introduction of quarantine organisms with WPM, the International Standard for the Use of WPM in International Trade was adopted in 2002 [5]. In accordance with the latest edition, exporters are offered four ways to decontaminate wood packaging:

- Heat treatment using steaming or oven for chamber drying (marking: HT);
- Heat treatment using dielectric heating (DH);

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- Treatment with methyl bromide (MB);
- Treatment with sulfuryl fluoride (SF).

Practice shows that not always enterprises producing WPM comply with this technical regulation or completely ignore it.

In a number of countries, most of the exported wood, including WPM, is disinfected with methyl bromide [9, 10], but this substance is known to deplete the planet's ozone layer. Intensive research is underway to find alternative fumigants for wood treatment. Ethanedinitrile (EDN) can be one of the promising and environmentally friendly substances for the disinfection of WPM. Research on the use of this fumigant against forest insects and nematodes is being actively conducted in the USA, New Zealand, the Czech Republic, and Russia [11, 12]. EDN is a promising analogue of methyl bromide for the disinfection of WPM, because it destroys not only insects in wood, but also pine wood nematodes *B. xylophilus,* the most resistant organisms to pesticides [13, 14].

Analysis of data on the registration of forest pests in the WPM when importing goods to European countries from around the world showed that the largest number of detections of organisms was in the WPM received from the Republic of Belarus (57, 92 and 73 detections, respectively, in 2017, 2018, 2019) and China (96, 52, and 56 detections), further from India (44, 36, and 23) and Ukraine (9, 29, and 7) (Fig.2). In the WPM from Russia, harmful organisms were detected 6 times in 2017 and 11 times each in 2018 and 2019.

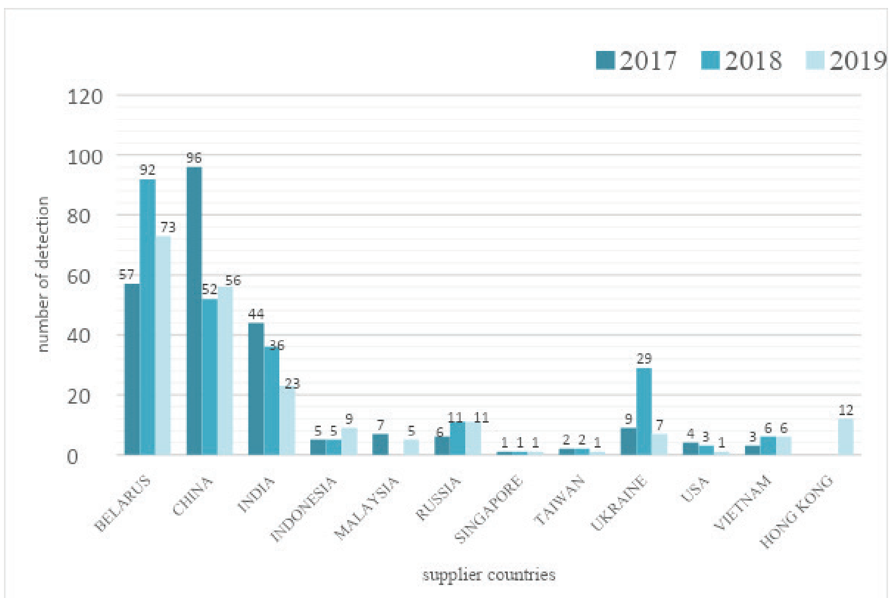

FIGURE 2. Number of detections of organisms (insects and nematodes) in wood packaging materials shipped with consignments from around the world to the EU, 2017-2019 (according to EUROPHYT)

Recently, there has been a tendency for the transit of a large volume of goods through the territory of Belarus, which is the reason for the significant number of detections of harmful organisms in the WPM supplied with goods from this country. China is the largest exporter of various products in the world, and a significant number of detections of pests and quarantine organisms in the WPM has always been characteristic of this country [15,].

Among the identified harmful organisms (Table1), the pine wood nematode *B. xylophilus* is among the most harmful and periodically detected. This organism poses the greatest threat to forest plantations, and can be introduced with WPM. When exposed to favorable conditions, the pathogen can cause mass wilting of coniferous plantations. Pest risk analysis has shown that the annual potential losses in forestry from the further spread of *B. xylophilus* for the EU are estimated at 300 million to 3 billion euros [16]. In the case of introduction of this nematode in Russia, the possible damage could amount to 47–112 billion rubles per year [17].

Below is the statistics of detections of the nematode *B. xylophilus* and the species *B. mucronatus*, which has a similar development cycle, for three years in the inspected WPM that arrived with cargoes to European countries (Table 1).

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B. xylophilus nematodes are periodically detected in the WPM with consignments from China and the USA, i.e. from regions where this pathogen is widespread. In 2018-2020 fewer cases of detection of *B. xylophilus* were registered than in the period 2014-2017 [17].

TABLE 1. Number of occurrences of *Bursaphelenchus xylophilus* and *B. mucronatus* nematodes in wood packaging materials shipped to Europe (according to EPPO and INTERNET EUROPHYT)

Pest	Exporting country	Country of discovery of the organism	Number of detections
2018			
B. xylophilus	Not detected		
B. mucronatus	Russia	Bulgaria	1
		Latvia	1
	Belarus	Belgium	3
		Germany	11
		Netherlands	2
		Lithuania	1
		France	1
	Ukraine	Lithuania	6
		Latvia	1
	China	Portugal	1
2019			
B. xylophilus	China	Poland	1
		Netherlands	1
	USA	United Kingdom	1
B. mucronatus	Russia	Lithuania	1
		Poland	2
	Belarus	Latvia	6
		Germany	12
		Belgium	1
		France	2
		Lithuania	1
		Netherlands	3
		Austria	1
		Estonia	1
		Poland	1
	Ukraine	Lithuania	2
		United Kingdom	1
	China	Germany	3
	Turkey	Lithuania	1
2020			
B. xylophilus	Not detected		
B. mucronatus	Belarus	Italy	1
		Latvia	1
		Lithuania	2
		Poland	1
		Germany	2
	Russia	Germany	1
	Turkey	Latvia	2

Table 1 also provides information on the detection of the nematode *B. mucronatus.* This species, unlike *B. xylophilus,* is widely distributed in Eurasia in coniferous plantations and is considered non-pathogenic. In addition to nematodes of the genus *Bursaphelenchus*, representatives of the genus *Aphelenchoides* are often found in wood. However, the presence of any species of live nematodes in the wood indicates that the WPM has not been subjected to phytosanitary treatment. In addition to nematodes, there is a threat of introduction with the WPM of longhorn beetles of the genus *Monochamus*, which are carriers of the quarantine species *B. xylophilus*. The registration of these pests is particularly important if the consignments originate from *B. xylophilus* distribution countries.

The Asian longhorn beetle *A. glabripennis* has been repeatedly found in the WPM in consignments from China (Table 2).

TABLE 2. Number of detections of the Asian longhorn beetle *Anoplophora glabripennis* in wood packaging materials shipped to Europe (according to EPPO and INTERNET EUROPHYT)

Date of detection of the pest (year)	Exporting country	Country of discovery	Number of detections
2018	China	Estonia	1
		EU	2
2020	China	Germany	1

* information about detection of *Anoplophora chinensis* in 2018-2020 is missing.

The country of origin of Asian and Chinese longhorn beetles *(Anoplophora glabripennis, A. chinensis)* is Asia. Back in the 1990s, the longhorn beetle *A. glabripennis* was introduced to the USA, Canada and some European countries (Italy, the Netherlands, Great Britain, France, Austria) with WPM from China. It causes significant damage to urban plantations of maple, plane tree. The Asian longhorn beetle belongs to heat-loving organisms, and it was assumed that its range in the Russian Federation could be limited to the Krasnodar Territory and the Republic of Crimea, however, the registration of the outbreak of this pest on a birch (*Betula pendula*) in Finland in 2015 [18] gave grounds to revise this forecast for the Russian Federation.

In contrast to the information on the registration of the Asian and Chinese longhorn beetles in the WPM for previous years [17], in 2018-2020 the number of the Asian longhorn beetle detections has decreased significantly.

Conclusion

Based on the statistics of detections of dangerous quarantine organisms in the WPM coming with consignments from abroad, it can be concluded that the application of the phytosanitary treatments recommended by ISPM No. 15 does not preclude additional verification by NPPO staff of these wood materials for compliance with the requirements of the international standard and the presence of they contain harmful organisms.

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Grain Bacterioses Phytosanitary Diagnostic as a Component of Food Security

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Abstract. The development of trade in agricultural products between countries requires improving the methods of regulating pathogens. This study was conducted in order to obtain data on the distribution of bacteria on grain crops in Russia, as well as to develop a strategy for optimizing methods of detection and identification phytopathogenic bacteria. Identification methods were found for 15 bacteria regulated on grain crops by the phytosanitary services of a number of countries by analyzing the literature sources. For the first time, as a result of phytosanitary surveys of more than 3,015 hectares of crops in four regions of Russia, 454 bacteria were isolated, some of which were identified. The obtained analytical and experimental data will allow updating data on the spread of phytopathogenic bacteria in Russia, developing methods for diagnosing grain bacterioses, and providing the phytosanitary service with methods for determining the compliance of Russian grain products with the requirements of importing countries.

INTRODUCTION

Solving the problem of eliminating hunger and ensuring food security is one of the main directions of UNO's strategic development. The improvement of technical regulation and the implementation of phytosanitary surveillance are among the criteria for ensuring the safety of agricultural products.

In recent years, there has been an increase in the aggressiveness of bacteria towards their host plants [1]. Not in all cases, the bacterium shows virulence. Most often, plants that are weakened by unfavorable conditions, cultivated with a violation of technology, suffer. Table 1 provides a list of bacteria associated with cereals. These species can cause economic damage directly, causing plant diseases and thereby reducing yields. They can also serve as an obstacle to trade relations, since all the bacteria presented are included in the phytosanitary lists of the importing countries of Russian grain.

TABLE 1. Grain crops bacterial pests that are important for plant quarantine

No.	Bacteria	Cereal host plants	Country	Phytosanitary status
1	*Acidovorax avenae*	*Avena* L., *Zea mays* L., *Oryza* L., *Fagopyrum esculentum* Moench and other cereals	Egypt East Africa, South Africa Morocco	Absent quarantine pest Restricted quarantine pest Quarantine pest
2	*Clavibacter tessellarius*	*Triticum* L.	Mexico	Quarantine pest
3	*Pectobacterium rhapontici*	*Triticum* L.	East Africa, South Africa, Brazil	Absent quarantine pest
4	*Pseudomonas cichorii*	*Triticum* L.	South Africa, Egypt	Absent quarantine pest

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			Jordan	Restricted quarantine pest
			Mexico	Quarantine pest
5	*Pseudomonas fluorescens*	*Secale cereale* L., *Triticum* L., *Sorghum* L., *Zea mays* L., *Oryza* L.	Mexico	Quarantine pest
6	*Pseudomonas fuscovaginae*	*Triticum* L., *Oryza* L., *Avena* L., *Hordeum* L., *Secale cereale* L., ×*Triticosecale* Wittm. & A. Camus, *Sorghum* L., *Zea mays* L., *Lolium perenne* L.	Egypt	Absent quarantine pest
7	*Pseudomonas syringae* pv. *atrofaciens*	*Triticum* L., *Hordeum* L., *Secale cereale* L., *Avena* L.	Egypt, Brazil	Absent quarantine pest
			Mexico	Quarantine pest
8	*Pseudomonas syringae* pv. *coronafaciens*	*Avena* L., *Secale cereale* L.	Mexico	Quarantine pest
			Egypt	Absent quarantine pest
			East Africa, South Africa	Restricted quarantine pest
9	*Pseudomonas syringae* pv. *syringae*	*Triticum* L., *Hordeum* L., *Secale cereale* L., *Avena* L.	Mexico	Quarantine pest
			Egypt	Regulated non-quarantine pest
			Jordan	Restricted quarantine pest
10	*Rathayibacter rathayi*	*Triticum* L., *Hordeum* L., *Secale cereale* L.	South Africa	Absent quarantine pest
			Tunisia, USA	Quarantine pest
11	*Rathayibacter tritici*	*Triticum* L.	Kazakhstan, Uzbekistan, Georgia, Moldova, Russia, Belarus, Armenia, Kyrgyzstan	Absent quarantine pest
12	*Xanthomonas translucens* pv. *cerealis*	*Triticum* L., *Hordeum* L., *Secale cereale* L., *Avena* L.	Mexico	Quarantine pest
13	*Xanthomonas translucens* pv. *graminis*	*Triticum* L., *Hordeum* L., *Secale cereale* L., *Avena* L.	South Africa, Brazil	Absent quarantine pest
			Israel	Quarantine pest
			Egypt	Included in the phytosanitary requirements
14	*Xanthomonas translucens* pv. *translucens*	*Triticum* L., *Hordeum* L., *Secale cereale* L., *Avena* L.	Egypt, Chile	Absent quarantine pest
			Jordan, Turkey	Restricted quarantine pest
			Morocco, Tunisia	Quarantine pest
			Nigeria	Prohibited for importation
15	*Xanthomonas translucens* pv. *undulosa*	*Triticum* L., *Hordeum* L., *Secale cereale* L., *Avena* L.	Nigeria	Quarantine pest

Pathogens of bacterial diseases of grain crops, except *Rathayibacter tritici*, are not regulated in Russia (Table 1). Therefore, it is possible to assess their distribution today only based on information from literature sources and

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databases. Identification in some cases was carried out by symptoms on plants, or using classical microbiology methods, without using molecular methods. Updating data on the distribution of bacterial species that are important for Russian grain exports could simplify trade relations between the countries.

Thus, there was a need to obtain data on the spread of bacterial grain crops pathogens, as well as to develop and optimize methods for their diagnosis. Effective methods of detection and identification can only be provided by a testing laboratory operating in accordance with the relevant international standards. The testing laboratory should use international regulatory documents for diagnostics, if available, as well as the methods described in the relevant scientific articles or journals. Methods developed by the laboratory or modified may also be used. In all cases, the laboratory must provide its production staff with a valid version of the diagnostic method, in which there are no discrepancies. Thus, if several diagnostic tests are available for a particular target bacterium, it is necessary to experimentally identify the most optimal tests in order to then include them in the diagnostic methodology [2].

Materials and Methods

Taking into account the relevance, a study was conducted, the purpose of which was to obtain data on the spread of bacterial grain crops pathogens in Russia, as well as to develop a strategy for optimizing methods for their detection and identification.

The tasks were solved in accordance with the set goal, as shown in the scheme (Fig. 1). This study is devoted to the following stages of the scheme: the collection of plant and seed material, the identification of bacteria in the collected material, as well as the collection and analysis of information about the pests and methods of their diagnosis.

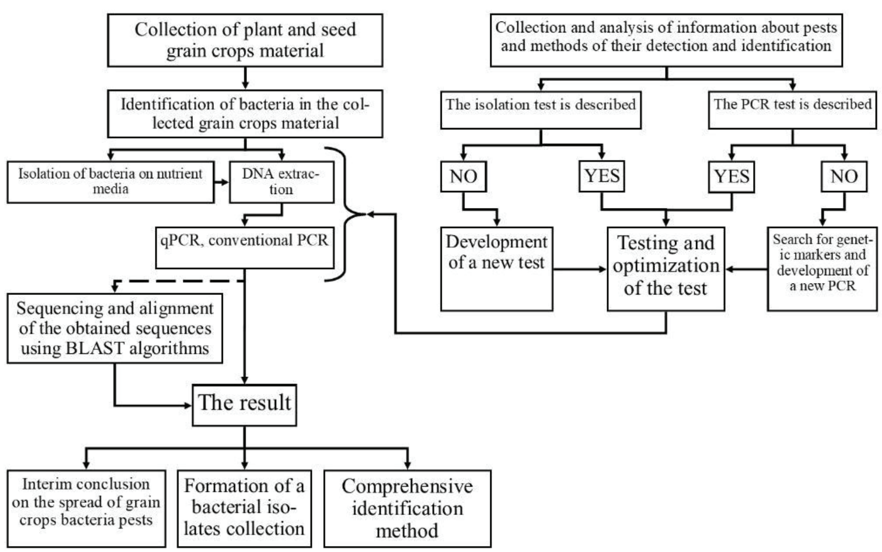

FIGURE 1. The scheme of work within the framework of the development of methods for the diagnosis of pathogens of bacterial diseases of grain crops. *Source:* Compiled by the authors

At the first stage of the work, we collected and analyzed information about the causative agents of bacterial grain crops diseases and methods of their diagnosis. First of all, the presence or absence of regulatory diagnostic documents was established. We also searched for scientific publications that describe methods that are applicable for routine diagnostics.

The next stage was devoted to the collection of plant and seed material in various regions of Russia. From each site occupied under one variety, one sample was taken.

Isolation of the bacteria was performed on media King's B, YDC, YPGA, NBY, and experimental CRL, CRL, mCRL media [3]. The isolated bacteria were identified by sequencing the PCR product and analyzing the resulting sequences using BLAST algorithms. PCR was performed with the primers SyD1/ SyD2, PSF/ PSR [4], and primers for 16S-23S rRNA on a T100 Thermal Cycler amplifier, "BioRad" (USA). A detailed description of the use of these PCRs for the identification of bacteria is given earlier [3].

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Results

The results of the search for diagnostic methods are presented in Table 2.

TABLE 2. Diagnostic tests for grain crops bacterial pests

Grain crops bacterial pests	Regulatory document	Isolation	PCR-RT, PCR
Acidovorax avenae	Absent	NA, SNR, Beef-Yeast extract [5], NBY, YDC, KB [6]	Oaf1/ Oar1 [5], Aaaf3/ Aaar2 and Aaaf5/ Aaar2 [6]
Clavibacter tessellarius	Absent	YDC, BCT medium [7]	PCR-RT CMT-F/ CMT-R and CMT-probe [8]
P. fuscovaginae	Absent	KB, NA [9]	PCR-RT *Pseudomonas fuscovaginae* by OOO “Sintol”, Russia; 6 primer pairs named Pf8 [9]
P. syringae pvs.	Absent	KB [10], SPTPsjA [11]	SyD1/ SyD2 [4]; PsyF/ PsyR [10]; rpoD-F/ rpoD-R, gyrB-F/ gyrB-R, cts-F/ cts-R, gapA230F/ gapA942R [12]
Pectobacterium rhapontici	Absent	PPGA [13]	isoRF/ isoR; TSU01/TSU02 and TSU03/TSU04 [13]
P. cichorii	Absent	KB, SPTPsjA [11]	PCR- RT PscHrc662F, PscHrc751R, probe PscHrcMGB687 [14]
P. fluorescens	Absent	KB [15]	16SPSEfluF/ 16SPSER [16]
Rathayibacter rathayi	Absent	YGM [17], NBY, NA, 523M [18]	FRATF3/ FRATF2 and probe FRATP1 [18]
Rathayibacter tritici	Present	YGM [17] NBY, NA, 523M [18]	5 primer pairs Rt-xF/ Rt-xR [19]; PCR-RT BTRITF2/ BTRITR2 and probe BTRITP1; PCR-RT BTRITF1/ BTRITR1 and probe BTRITP1 [18]
X. translucens pvs.	Absent	WB, NBY [20], YDC, KM-1, WB Boric Acid-cephalexin, XTS [21]	PCR for housekeeping genes proD, dnaK, fyuA and gyrB [20]

NA – Nutritive agar; YDC – Yeast extract-Dextrose-Calcium Carbonate; SNR medium – Sorbitol Neutral Red medium; KB – King B medium; SPTPsjA – serine-potassium tellurite-based Psj-selective agar; PPGA – potato-peptone-glucose agar; NBY – nutrient broth with yeast extract

Recommended culture media, as well as PCR tests, were found in scientific publications for all the studied bacterial species.

The results of the phytosanitary surveys are presented in Table 3.

TABLE 3. Results of phytosanitary surveys

Area	Crop	Number of varieties	Number of plots	Identified bacteria	Number of isolates
Sverdlovsk region, production plots, 2019					
89 ha	Winter barley	1	4	*Pseudomonas* sp., *Bacillus* sp., *Curtobacterium* sp., *Paenibacillus, Pseudomonas azotoformans, Pantoea agglomerans*	15

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138 ha	Winter wheat	1	6	*Pseudomonas* sp., *Bacillus* sp., *Stenotrophomonas, Pseudomonas syringae, Curtobacterium* sp.*, Pseudomonas hibiscicola,*	23
Rostov region, production plots, 2019					
18 ha	Spring barley	1	1	*Pseudomonas* sp., *Pantoea agglomerans, Enterobacteriaceae, Paenibacillus, Stenotrophomonas, Bacillus* sp., *Erwinia* sp., *Pantoea* sp., *P. syringae* pv. *syringae, P. syringae* pv. *atrofaciens,*	18
166 ha	Winter barley	3	3	*P. azotoformans, Pseudomonas* sp. *Paenibacillus, Stenotrophomonas, Bacillus* sp., *Erwinia* sp., *Pantoea* sp.	24
1536 ha	Winter wheat	14	18	*P. fluorescens, P. poae, Pseudomonas* sp., *P. curtobacterium* sp., *P. hibiscicola, Paenibacillus, Stenotrophomonas, Bacillus* sp., *Erwinia* sp., *Pantoea* sp., *P. syringae* pv. *syringae, P. syringae* pv. *atrofaciens*	74
Moscow, hybridization plots, 2020					
47 m^2	Winter wheat	47	47	*Actinobacterium* sp., *Agreria* sp., *Arthrobacter* sp., *Bacillus* sp., *Cellulomonas* sp., *Clavibacter* sp., *Curtobacterium* sp., *Erwinia* sp., *Frigoribacterium* sp., *Frondihabitans* sp., *Kineococcus* sp., *Lysinimonas* sp., *Microbacterium* sp., *Micrococcus* sp., *Oerskovia* sp., *Pantoea* sp., *Phycicocous* sp., *Plantibacter* sp., *Pseudomonas* sp., *Rahnella aquatilis*, *Rhodococcus* sp., *Salinibacterium* sp., *Sanguibacter* sp., *Sphingomonas* sp., *Streptomyces* sp., *Subtercola pratensis*	164
1 m^2	Winter rye	1	1	*Pseudomonas* sp., *Rhodococcus* sp.	3
1 m^2	Spring rye	1	1	*Rhodococcus* sp., *Pseudomonas* sp., *Pseudoclavibacter helvolus*	5
6 m^2	Winter triticale	6	6	*Clavibacter michiganensis*, *Curtobacterium flaccumfaciens*, *Dyadobacter* sp., *Frigobacterium faeni*, *Pseudomonas veronii*	6
Republic of Crimea, production plots, 2021					
466 ha	Winter wheat	4	13	*Actinobacterium* sp., *Arthrobacter* sp., *Bacillus* sp., *Clavibacter* sp., *Curtobacterium* sp., *Erwinia billingae*, *Frigoribacterium* sp., *Microbacterium* sp., *Micrococcus* sp., *Oerskovia* sp., *Pantoea agglomerans*, *Phycicocous* sp., *Pseudomonas* sp., *Rahnella aquatilis*, *Rhodococcus* sp., *Sphingomonas* sp., *Streptomyces* sp.	39
454 ha	Winter barley	4	10	*Clavibacter* sp., *Curtobacterium* sp., *Erwinia* sp., *Microbacterium phyllosphaerae*, *Pantoea agglomerans*, *Pseudomonas* sp.	11
16 ha	Oats	1	1	*Arthrobacter* sp., *Pseudomonas* sp.	3
132 ha	Cereal mix	3	2	*Erwinia* sp., *Pantoea agglomerans*	4
Republic of Crimea, plots of variety testing, 2021					
400 m^2	Winter wheat	5	16	*Actinobacterium* sp., *Clavibacter* sp., *Curtobacterium* sp., *Erwinia* sp., *Frigoribacterium* sp., *Microbacterium* sp., *Pantoea agglomerans*, *Sphingomonas* sp.	14
300 m^2	Winter barley	6	12	*Arthrobacter* sp., *Bacillus* sp., *Clavibacter* sp., *Curtobacterium* sp., *Erwinia* sp., *Frigoribacterium* sp., *Microbacterium* sp.,	23

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150 m^2	Oats	6	6	*Micrococcus* sp., *Pantoea agglomerans*, *Pseudomonas* sp., *Rhodococcus* sp., *Sphingomonas* sp. *Bacillus* sp., *Microbacterium phyllosphaerae*, *Pseudomonas* sp., *Rahnella aquatilis*, *Streptomyces* sp.	8

Discussion

It is established that the regulatory document on detection and identification is available only for *Rathayibacter tritici* (Table 2). It should be noted that this method does not describe the PCR tests. The lack of the possibility of PCR identification of the target bacterium by this method reduces its relevance.

For *Acidovorax avenae*, *Clavibacter tessellarius*, *P. fuscovaginae*, *P. cichorii*, *R. rathayi*, and *R. tritici*, a description of real-time PCR (PCR-RT) is found in the literature (Table 2). PCR-RT is the most preferred diagnostic test for routine diagnostics due to minimal time and labor costs [22], so tests based on PCR-RT are subject to assessment of applicability first.

At least one PCR test was found for each target bacterium (Table 2). Most PCR tests, according to the authors, are species-specific, which suggests that there is no need for subsequent sequencing. However, in the process of evaluating the applicability of PCR in testing a large number of closely related bacteria, results may be obtained that may impose restrictions on the use of tests. For example, when testing PSF/ PSR primers to identify the species *P. syringae* [4], PCR products were obtained for all bacteria of the genus *Pseudomonas* [3]. Each PCR test listed in Table 2 is subject to testing.

No species-specific PCR tests were found for pathogens of the species *X. translucens*. There is a source that has published primers for PCR and subsequent sequencing to determine the pathway [20]. The sequencing results, due to the use of the NCBI database for analyzing the obtained sequences, can be used for scientific purposes, but their use for diagnostics by an accredited laboratory in the analysis of a sample is not applicable due to the low degree of confidence. Thus, the development of species-specific PCR tests for *X. translucens* is required.

Recommendations for isolation on culture media were found for each target bacterium (Table 2). Among the proposed media options, it is necessary to experimentally identify the most optimal one.

Surveys were conducted on an area of more than 3015 ha of wheat, barley, triticale, oats and rye crops in the Sverdlovsk and Rostov regions, Moscow and the Republic of Crimea (Table 3). 434 bacteria were isolated, some of which were identified, while others are in the process of determining their species. A comparison of the experimental data obtained with the information on the distribution of bacterial cereals pathogens, given in the literature, showed: none of the isolates from the samples of cereals collected in Moscow, is not defined as belonging to the genus *Xanthomonas*, while the Crop Protection Compendium (CABI) describes that *X. translucens* pv. *cerealis* and *X. translucens* pv. *translucens* are distributed in the Central part of Russia. According to CABI, *P. syringae* pv. *atrofaciens* distributed only in Siberia, however, strains of this bacterium were isolated from wheat and barley plants after surveys of grain crops in the Rostov region (Table 3). *P. syringae pv. syringae* is common in Western Siberia and the Far East, but strains of the bacterium were also found in the Rostov region (Table 3). *P. syringae* pv. *coronafaciens* is distributed in the European part of Russia, in Eastern and Western Siberia, and *P. cichorii* and *P. fluorescens* are widely distributed throughout Russia. However, the methods used in this study only detected *P. fluorescens* isolates on rye and winter wheat crops in Moscow and on winter wheat crops in the Rostov region (Table 3). The remaining bacteria, listed in Table 1, were not identified in this study. *Pseudomonas* bacteria, whose species has not yet been identified, can be identified by testing and implementing species-specific PCR tests (Table 2).

Conclusion

This study was conducted in order to obtain experimental data on the spread of bacterial grain crops pathogens, as well as to develop a strategy for optimizing methods for their detection and identification. Within the framework of this goal, such tasks as conducting surveys of grain crops in various regions of Russia, identifying bacteria in the collected material, as well as searching for literature sources describing methods for detection and identification target bacteria were solved.

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As a result, the identification of bacteria isolated from grain crops of various varieties and origin was carried out. The obtained data allowed us to conduct a comparative analysis with the literature data on the distribution of bacteria. All the isolates can be used in further work during the approbation and validation of diagnostic tests.

The data on the existing diagnostics methods of grain crops bacterioses are analyzed. Cultural-morphological and molecular-genetic tests were found, the description of which allows us to conclude about their possible applicability in routine diagnostics. The found diagnostic tests are further subject to an assessment of their applicability, according to the results of which the most optimal of them will be included in the methodology for detection and identification grain bacterial pathogens of grain crops.

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Soil Oomycetes Associated with Plants of the Genus Juglans in the European Part of Russia

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Abstract. The potential of the southern regions for the cultivation of nut crops in Russia is currently not used in full. This is despite the stable growth in consumer demand for products in this segment. The climatic conditions of the Southern and North-Caucasian Federal Districts make it possible to grow nut crops on an industrial scale. However, the establishment of new plantings is limited by the availability of high-quality planting material for target crops of domestic selection. Most of the new plantings are carried out with imported planting material. Seedlings with a closed root system, where soil pathogens can persist in latent form, pose a particular danger. Visually showing no symptoms at the initial stage of the lesion, in the future, these pathogens can lead to significant crop losses. The purpose of the research was to study the species diversity of soil oomycetes associated with walnut crops. The objects of the study were plants of the genus Juglans with symptoms indicating root lesions. The preliminary identification of pathogens was carried out by cultural and morphological methods, precise identification up to the species – by decoding the nucleotide sequences of diagnostically significant regions: the internal transcribed spacer (ITS) and the ras-related protein gene (Ypt1). As a result of the research, 7 species of micromycetes were identified, belonging to 2 orders, 3 families of the Pseudofungi division (fungus-like organisms, "pseudo-fungi"). It was found that the largest number of species of soil oomycetes is associated with Juglans regia plants. Significantly fewer species were identified in soil samples taken from Juglans nigra.

INTRODUCTION

The genus *Phytophthora is* one of the largest in the order *Peronosporales* of the Oomycetes class and includes more than 180 described species, which differ in the degree of pathogenicity and harmfulness [1]. Soil fungus-like organisms from the genus *Phytophthora* infect a wide range of host plants, cause root and root rot, leaf burn, shoot death, bark necrosis, fruit rot, wilting, ulcerative and other lesions. The causative agents of late blight form mobile zoospores, which allow them to spread in water and waterlogged soil and, especially, easily with streams of water during irrigation and heavy rains. Sexual structures of late blight – oospores – are transferred with soil particles on agricultural implements or machines, with roots. Oospores can remain viable in soil for a long time [2].

Over the past few decades, interest in these dangerous phytopathogenic oomycetes has increased markedly. This was facilitated by the introduction of molecular genetic methods of identification, which made it possible to identify many new species, expanding the range of affected host plants in the long described species. New approaches and the use of modern diagnostic methods have led to an increase in the number of taxa identified in this genus, from about 58 in 1996 [2] to more than 150 species in 2017 [1].

Timely diagnosis of late blight pathogens is fundamental to control and prevent their spread [3]. An important step in this work is the isolation and precise identification of phytopathogens. Different approaches have their own advantages and disadvantages. Direct isolation of *Phytophthora* spp. from the soil is hindered by the biological characteristics of these pathogens. They have a rather slow growth and do not withstand competition in crops on nutrient media with fast-growing and numerous micromycetes. Selective media for the isolation of pathogens, as a rule, consist of corn agar (CMA) or V8 agar with different combinations of antibiotics: pimaricin, ampicillin, rifampicin, vancomycin, nystatin; and fungicides: pentachloronitrobenzene (PCNB) and hymexazole (also known as

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Tachigaren). The selective action of these agents is provided mainly by pimaricin or nystatin, since they are active against most fungi. Gimexazole inhibits most *Pythiumand* and *Morteriella* species, which can inhibit and mask *Phytophthora*. The combination of rifampicin and ampicillin is more effective in suppressing bacteria than vancomycin.

A wide range of methods have been reported for the successful isolation of *Phytophthora* from soil and water samples, including direct seeding of soil onto media and the bait method. Due to the limited amount of soil that can be applied to the surface of the medium, direct seeding is usually used when the soil is heavily infested with *Phytophthora*. The bait method has several advantages over direct seeding. First, a larger volume of soil can be tested, which increases the likelihood of detecting species present in the sample. Second, homothallic species that persist as dormant oospores are more likely to be detected by bait techniques than by direct seeding.

Most of the molecular genetic methods for the identification and detection of oomycetes of the genus *Phytophthora* have been developed on the basis of the internal transcribed spacer (ITS) of ribosomal DNA. [4]. At present, the ITS sequences of almost all types of late blight have been studied. According to many researchers, this region of the genome is sufficiently variable to distinguish root rot pathogens at the species level [5, 6]. Nevertheless, Kroon L.P.N.M. (2004) et al. [7] noted that in the diagnosis of closely related species of *Phytophthora* spp. the sequence of this site proved to be insufficiently variable. For interspecies difference, it became necessary to conduct additional analysis of amplification fragment length polymorphism (AFLP) [6]. Among the targets in the genome of *Phytophthora* spp. used in molecular studies, amplification of the ras-related protein (Ypt1) gene fragment turned out to be the most promising for species identification within the genus [8]. The high polymorphism of this region is of great importance for the differentiation of closely related species, which are characterized by almost identical sequences of ITS regions [7]. The availability of data on parts of the genome of late blight species makes it possible to develop modern methods for determining them, and at present there are molecular methods for identifying the pathogen at any stage of its development.

On walnut trees late blight causes damage to the roots, the lower part of the trunk, leads to the death of the affected tissues and, as a result, to the death of trees at different ages. On *Juglans regia*, several species of *Phytophthoraare* described, including *P. cactorum, P. citricola* and *P. citrophthora*. In Italy, the species *P. gonapodyides* was isolated from walnut plants with strong leaf wilting, crown sparseness, and root collar rot [9]. In Turkey, sudden wilting and subsequent death of about 15% of *J. regia* trees was associated with the defeat of plantations by *P. chlamydospora*, previously known as the Pgchlamydo taxon [10]. Currently, phytopathogenic fungi on walnut crops have been studied to a limited extent. In the available domestic literature, there are isolated reports on spotting, branch diseases, and cancerous lesions of *Juglans* plantations in the Non-Chernozem zone of central Russia [11, 12].

All this determines the need for ongoing research. The purpose of which was to study the species composition of soil-dwelling oomycetes under different types of walnut (genus *Juglans),* with symptoms of damage to the root system. The tasks of the research included conducting phytosanitary surveys of walnut plantations, taking soil samples, followed by isolating pathogens, identifying them by cultural-morphological and molecular-genetic methods, for the presence of late blight pathogens and their identification.

Materials and Methods

The study was carried out in 2020 in the Michurinsky garden of the Russian State Agrarian University-Moscow Agricultural Academy named after K.A. Timiryazev, laid on sod-podzolic soil. A total of 29 soil samples taken under plants of walnut (*Juglans regia*), Manchurian walnut (*Juglans mandshurica)*, Lancaster walnut (*Juglans cordiformis* x *cinerea*), ailanthus-leaved nut (*Juglansailantifolia* var. *cordiformis*), black walnut (*Juglans nigra*), which had symptoms of drying out, wilting and ulcerative lesions of the stem, slowing growth and wilting, with elongated necrosis on the trunk and shoots.

For analysis from one site from the tree under study, point samples were taken, from which the combined samples were made. Each combined sample consisted of three to five point samples weighing from 200 to 250 g, taken from a depth of 10-20 cm, near the plant roots.

Isolation of representatives of the genus *Phytophthora* from soil samples was carried out by two methods – surface sowing of soil suspension on a selective nutrient medium P5ARP [H] and by the method of floating biological baits with subsequent isolation of pathogens on the medium – P5ARP [H]. After incubation of the plates at 24 °C, they were examined and the growth of colonies, characteristic of late blight pathogens, was noted, and they were subcultured onto agar with carrot pieces.

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Walnut leaves were used as bio-baits, which were laid on soil samples according to the method of S.E. Golovin. (2001) [13]. Plates with bioframes were incubated at 22–24 °C for 5 days. For further studies, small fragments were cut from the affected bait leaves with the capture of healthy and infected tissue and placed on a selective nutrient medium P5ARP [H] (link), after the appearance of growth, the mycelium was transferred to agar with carrot pieces.

For species identification, well-visible light colonies with irregular edges and concentric rings characteristic of late blight oomycetes were selected. DNA was isolated from the mycelium using the "MetaGen" kit made by "Syntol", Moscow.

Classic PCR was performed with primers ITS1/ITS4 [14], which amplified a 900 bp product of the ITS-5.8S rDNA region and YPh1F/YPh2R [15], which amplified a 470 bp product of the ras-related protein gene fragment. The samples were sequenced according to the Sanger method. The sequencing reaction was performed using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) according to the manufacturer's instructions, followed by separation of the fragments on an ABI PRISM 3500 automatic genetic analyzer (Applied Biosystems).

Results and Discussion

As a result of the studies, oomycetes, representatives of genera *Phytophthora*, *Pythium* were identified in soil samples under different tree species of genus *Juglans* (Table 1).

TABLE 1. Soil oomycetes in sod-podzolic soil under plants of the genus *Juglans* (Moscow)

Plants	Signs of the disease	Number of soil samples	Number of isolates obtained	Identified species
Juglans regia	Leaf burn, leaf wilting, twigs dieback, crown wilting	17	2	*Phytophthora cactorum*
			1	*Pythium heterothallicum*
			1	*Pythium torulosum*
			1	*Pythium diclinum*
Juglans nigra		3	1	*Phytophthora plurivora*
Juglans mandshurica		2	-	-
Juglans ailantifolia var. *cordiformis*		2	-	-
Juglans cordiformis x *cinerea*)		1	-	-

The largest number of isolates of soil oomycetes of the genus *Phytophthora and Pythium* were isolated on walnuts. Of the 17 studied samples, root rot pathogens were found in 29.4% of cases. The dominant species in the studied samples of *Juglans regia* was *P. cactorum*. Also, representatives of the genus *Phytophthora* were found in a single case on a black walnut – *P. plurivora*. In soil samples of *Juglans mandshurica, Juglans ailantifolia var. cordiformis, Juglans cordiformis x cinerea*, representatives of the *Pseudofungi* division were not found. Pythium pseudo mushrooms were isolated only from walnut soil samples.

In different countries, the economically significant species on walnuts are *P. gonapodyides, P. cinnamomi, P. citrophthora, P. chlamydospora*. In California, a complex of Phytophthora species, including *P. citricola, P. cactorum, P. cinnamomi, P. citrophthora, P. megasperma,* and *P. cryptogea,* were found to cause root rot in black walnuts, resulting in trees death [16].

Supervision of surface sowing revealed a significant variety of fungal colonies, differing in growth rate and mycelium character and other cultural properties. Among them and the colonies weeded out from the leaves of the walnut used as bait, colonies with characteristic morphological features of oomycetes were identified. On nutrient

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medium P5ARP [H], they had white, cobweb, chrysanthemum-like mycelium. As a result, colonies similar in cultural and morphological characteristics to *P. cactorum, P. plurivora* and *P. citricola* were obtained. Isolates of *P. cactorum* were isolated most easily in a pure culture. It should be noted that, according to studies by other authors, this type of late blight is most often associated with root collar rot, necrotization of walnut roots [9, 10, 16].

On carrot agar, colonies of *P. cactorum* are whitish in color, clearly visible, reaching a diameter of 70 mm on the 7th day. Mycelium is adpressed, creeping, white. Sporulation is present. The colonies are compact, slightly radiant, without a clear border, not dense with a homogeneous slightly airy mycelium. The optimum growth temperature was 20-25 °C, the culture did not grow at temperatures above 31 °C.

The sporangia of *P. cactorum* were oval or lemon-shaped, inversely pear-shaped, rarely asymmetrical. Oospores are spherical, 20-30 μm in diameter, colorless and yellowish, with a shell 1-2 μm thick. Chlamydospores were formed in large numbers, spherical, colorless or yellowish, 19-53 μm in diameter. It is one of the few pathogens of late blight with a wide range of affected plants – more than 250 species. On the territory of Russia, this species is most often observed in strawberry plantations, where it causes fruit rot and root rot. Also, *P. cactorum* is the main cause of rootstocks and seedlings falling out in nurseries and late blight rot of the root collar of apple trees and pears of already mature plantations [17].

The shape of the *P. citricola* sporangia varied. They were obovate, obovate-clavate, pear-shaped or slightly flattened on one side, and less often deeply bifurcated with two apices or irregular in shape. The sporangia were non-falling and persistent on the stalk. The semi-papillary papillae were wide and flat. Sporangiophores were simple or sometimes sympodial.

The sporangia of *P. plurivora* usually formed terminally on unbranched sporangiophores. Hyphalic swellings were sometimes noted, which formed in the nodes. The sporangia were non-falling, semi-papillary, rarely with two or three papillae with a well-defined basal fork. Previously, we identified this species in soil samples taken from oak and maple in the city of Pyatigorsk, as well as in the city of Kislovodsk in soil samples from chestnut and maple.

P. plurivora is part of the *P. citricola* complex together with *P. multivora, P. pini, P. capensis, P. citricola.* A description of the cultural and morphological characteristics that distinguish *P. plurivora* from *P. citricola* are presented in Burgess and Young (2009). These are the ratio of the length and width of *P. plurivora* sporangia, differences in morphological structures, the nature of the growth of colonies on Potato dextrose agar, and faster growth of *P. plurivora* on Potato dextrose agar at 20 °C and on V8 at 20-30 °C.

Cultural and morphological identification of the obtained isolates was confirmed by deciphering the nucleotide sequence of the internal transcribed spacer (ITS) of the Ypt1 gene. Analysis of the nucleotide sequences showed 100% identity with the published sequences available in GenBank: *P. cactorum, P. plurivora and P. citricola*.

As a result of the research carried out, representatives of the genus *Phytophthora*, and the related genus *Pythium,* were isolated only in the samples obtained using the bio-bait method. In the samples obtained by the method of surface sowing of soil suspension, the overwhelming majority of the genus *Gongronella* was revealed, and the species *Mucor sp, Absidia sp, Clathrina sp*. This is due to the fact that the soil contains a small amount of propagules of representatives of the genus *Phytophthora*, in comparison with the accompanying mycoflora, which suppressed the growth of oomycetes.

Conclusion

The results of studying the species composition of soil oomycetes show that representatives of the genera *Phytophthora*, *Pythium* of the *Pseudofungi* division are associated with the species of the genus *Juglans* in the European part of Russia. The most susceptible to soil oomycetes were the gray walnut plants, on which the largest number of species of mushroom-like organisms was found. The method of floating bio-baits in combination with sowing on a nutrient medium is the most effective for isolating late blight, in comparison with the method of direct surface sowing of soil suspension. A more reliable diagnosis requires a combination of both classical identification methods and molecular genetic diagnostic methods. Moreover, when decoding nucleotide sequences, it is necessary to use at least two variable regions (in our studies ITS, Ypt1), which significantly increases the reliability of the results obtained. All soil oomycetes identified in these studies were first described in Russia as associated with plants of the genus *Juglans.*

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Insect Pheromones as an Indispensable Component of Organic Agriculture

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Abstract. The article formulates current ideas about the role of synthetic insect pheromones in ensuring the ecological safety of agricultural production, forest protection, and environmental protection. The place of pheromones in the integrated plant protection system is substantiated; the main advantages of pheromones compared to traditional chemical insecticides and potential directions of the practical application of artificial pheromones are indicated.

Introduction

In recent decades, the concept of an integrated plant protection system has been formed and successfully developed in the science and practice of agriculture and forestry, which provides for the rejection of the widespread use of potent chemicals with their partial or complete replacement by the integration of other safer methods of pest control available to the modern agro-industrial complex. Currently, the main emphasis is not on the extermination of all harmful organisms by any means, but on a safe, effective, and economically justified reduction in the pest population, i. e., on managing their harmfulness [1, 2].

The use of pheromones, biologically active substances produced by insects themselves to transmit information to individuals of their species to modulate their behavior, fits into this concept perfectly. Synthetic sexual and aggregation pheromones of insects have been widely used in the developed countries of the world in the integrated plant protection system as one of the main means of monitoring and reducing the number of pests for about half a century [3-7]. Interest in the study of pheromones began to grow especially intensively in the last years of the twentieth century when the development of more and more advanced means and methods of complex pest control has begun on their basis. The same trend continues at the beginning of the new twenty-first century, which is confirmed by the growing interest of foreign partners in Russian-made pheromones.

Results and discussion

Advantages of pheromones

The study of insect chemocommunication has shown that pheromones are specific to each species. The use of synthetic pheromones disrupts the most important vital processes of pests without any effect on other living organisms. This selectivity is especially important for the preservation of useful species in agrocenoses, primarily entomophages because traditional insecticides, as a rule, are not selective and can have the same detrimental effect on all species – harmful and useful, targeted and non-targeted. Unlike pesticides, the effects of pheromones are not toxic, and therefore their use does not pose any risk to the environment. The results of toxicological studies of pheromones have shown that their toxicity to warm-blooded animals, fish, and plants is extremely low in comparison with conventional pesticides [8]. Pheromones are among the strongest of all known biologically active

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substances. For example, 1 mg of the synthetic pheromone of gypsy moth in the field retains an attractive effect on the pest for three months and allows monitoring the state of its population at a distance of up to several kilometers. By being products of natural origin, pheromones are secreted by insects in nanogram quantities and are perceived in the amount of several molecules. The use of synthetic pheromones does not require the creation of large-scale productions, since up to several milligrams of pheromone are used in monitoring and mass trapping, and in case of disorientation, the amount of pheromone does not exceed several tens of grams per hectare. To obtain such a number of pheromones, their synthesis can be carried out in the laboratory. Neither natural nor synthetic pheromones can accumulate in the environment – they decompose quite easily and quickly under the influence of sunlight, temperature, and humidity. Thus, taking into account the species specificity, the absence of toxicity to humans and animals, and the opportunity of use in ultra-small quantities, pheromones as a plant protection agent are the safest for the environment [8].

Practical application of pheromones: ways and problems

The practical importance of pheromones in the system of modern integrated pest protection in agriculture and forestry cannot be overestimated.

The use of pheromones is necessary, first, to identify and monitor the spread, study the dynamics of the number and density of the pest population. Traps with synthetic pheromones make it possible to speed up and facilitate the monitoring process, by increasing its productivity and quality exponentially. To do this, several traps for tens (and sometimes hundreds) of hectares are enough, which makes pheromones simply irreplaceable in fruit orchards [9] and on a huge area of Russian forests [3, 10, 11]. To date, this is the most cost-effective way to detect and assess the number of pests compared to other known methods, as it makes it possible to determine the localization and scale of pest foci, predict the timing of their occurrence, study the seasonal activity of the pest and, as a result, clearly determine the timing and volume of insecticidal treatments, reduce their number, significantly increase their targeting and effectiveness and thereby reduce the negative impact of hazardous chemicals on the environment and ensure high quality and environmental safety of agricultural products. The competent use of pheromone traps makes it possible to reduce the use of insecticides by 40-70% [8, 9].

If the pest population density is low, but above the economic threshold of harmfulness, it is possible to control the pest with the help of a pheromone. The fight is carried out by the method of mass trapping or by the method of disorientation.

During mass trapping, traps, being located linearly along the perimeter of the protected plantation, can perform a barrier role [3]. Being located inside the protected plantings, traps can interfere with the normal mating of pests, by creating the effect of a "male vacuum" [12]. And here it is important to note that pheromone dispensers placed in attracting traps in no way come into contact with surrounding living organisms (except for target pests) and with protected agricultural products and, consequently, the use of pheromones for mass trapping is possible throughout the growing season, regardless of harvest timing [8]. Often, to enhance the effect, synthetic pheromones and attractants are used in combination with color attractive traps or traps together with chemical or biological insecticides or chemosterilants (the so-called "attract and kill" technology means "attraction and destruction") [13]. And in this case, the contents of the trap do not come into contact with either the crop or the environment and are easily removed at the end of the period of action together with the trap, and the amount and doses of insecticides used are minimal. Thus, pheromones can be applied in parallel with almost any other plant protection product – with biomethode, bioinsecticides, or chemical insecticides.

When using the disorientation method, the mating disorder occurs due to the saturation of the air with pheromone throughout the protected area. Such an effect can be achieved by placing many pheromone sources in agricultural plantations, which can be easily removed later or by spraying micro drops or microcapsules containing pheromone from the air [4, 7, 14]. An interesting development is a method of "autoconfusion", which has found the greatest application in the fight against click beetles (Coleoptera: Elateridae). At the same time, an electrostatic powder containing a sexual pheromone is applied to males of the laboratory pest population at the time of their release into natural habitats. Once free, such "smelling female" males not only lose their ability to mate normally but also disrupt the mating process in the entire wild population [15].

It is especially necessary to emphasize the crucial role of pheromones in such specific areas as protection from quarantine pests and stock pests.

The number of quarantine pests in the early stages of introduction is usually small, and it is necessary to detect and destruct them as soon as possible. Therefore, pheromone methods with their high sensitivity and selectivity are

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ideal for solving this problem. Currently, pheromones of many quarantine species have been already successfully used in practice [16-18]. For example, with the help of pheromone traps, Rosselkhoznadzor specialists repeatedly intercepted a corn beetle that was absent from the Russian territory along the border with Ukraine [16].

The use of chemical means of pest control of agricultural stocks in the food industry is very dangerous for the health of the consumer and therefore is very limited. And here, in a confined space, pheromones have practically no alternative. In particular, pheromone traps have been successfully used for many years to combat mill and barn moths at some Moscow bakeries [19].

By the beginning of the twenty-first century, pheromones have already been studied in more than 700 species of insects. In the USA, for example, about 200 pheromones of the main pests of agriculture and forestry are used for monitoring and mass trapping, 26 pheromone preparations are used for disorientation, and their number approximately corresponds to the number of insecticidal drugs used in the USA. More than a hundred different pheromones are successfully used in Hungary, Bulgaria, and other European countries with traditionally highly developed agriculture. Pheromones are also widely used in Canada, Australia, China [1, 2, 5, 18, 20, 21]. This scientific and practical direction needs accelerated development in our country following the requirements of modern ecology, agriculture, and forestry.

Due to the great importance of this issue for ensuring food, environmental and phytosanitary security of the state in most developed countries of the world, the basis for the introduction of pheromones into agricultural practice is state support for scientific research, mass production, and widespread use of pheromones in the form of national targeted programs. In 2008, at the initiative of the Rosselkhoznadzor, a department for the synthesis and application of pheromones was created in the FSBI All-Russian Centre of Plant Quarantine to provide the phytosanitary service of Russia with pheromones of quarantine pests. Currently, this department produces pheromones of more than 60 types of quarantine and especially dangerous non-quarantine insect pests of agriculture and forestry. For more than ten years, Rosselkhoznadzor has been successfully implementing the “Program (Plan) for the detection of quarantine pests on the territory of the Russian Federation using pheromone and color traps in phytosanitary risk zones” developed by specialists of the FSBI All-Russian Centre of Plant Quarantine.

One of the main reasons preventing the widespread use of pheromones in agriculture and forestry is the attribution in the territory of the Russian Federation and the EEU countries of these analogues of natural substances to chemical plant protection products, the registration of which at the moment still requires a lot of time and financial costs. This contradicts the entire world experience in the use of pheromones. According to information from the official website of the USA Environmental Protection Agency, for example, pheromones and identical or substantially similar compounds labeled for use only in pheromone traps are not subject to regulation. Simplified approaches to pheromone registration are used in Canada, Argentina, Chile, Greece, Hungary, and other countries with developed agriculture. Leading specialists of scientific institutions engaged in the study and application of insect pheromones have repeatedly appealed to the highest instance with a reasonable proposal to cancel the procedure for registering pheromones as insecticides on the territory of Russia and the EEU countries, but, unfortunately, this issue has not received a positive decision.

Conclusion

In conclusion, we can say: following the priority directions of the Strategy of scientific and technological development of the Russian Federation (p. 20 “g”) system of integrated plant protection in all its diversity, including synthetic insect pheromones as an equal and essential component, opens the agro-industrial complex of large capacity to ensure high yield and high quality of agricultural products and, at the same time, to ensure the environmental safety of production and consumption. “Ecological agriculture” and “organic products” are not hollow words (unless, certainly, we leave aside pure populism and pure advertising). Modern science and modern agricultural production have all opportunities to fill these concepts with practical meaning and achieve practical results in this area. Certainly, solving these problems requires a competent approach from the agricultural producer and is not always easy and cheap. Nevertheless, the implementation of these solutions in a civilized economically developed state is quite possible – after all, the ultimate goal of the entire program is people’s health.

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Phytosanitary Problems of Organic Farming and the Use of Biological Control Agents in Agrotechnological Charts of Organic Production Processes

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Abstract. This article examines the main problems of organic production in the world and in the Russian Federation. It is shown that the main method of protecting plants from pests and pathogens is the biological method. It is noted that the most important condition for long-term and mutually beneficial cooperation of all the world countries in the field of organic farming is compliance with strict organic standards, which primarily include agrotechnological charts. Agrotechnological charts include all the necessary measures for the biological system of protection plants from diseases, pests and weeds and are part of the transparency plan of production processes.

INTRODUCTION

In the Russian Federation, starting from January 1, 2020, the Federal Law "On Organic Products and on Amendments to Certain Legislative Acts of the Russian Federation" (No. 280-F3) came into effect. This document establishes the regulation of the organic products market and establishes the basic concepts: "organic products", "organic agriculture", "producers of organic products"; introduces a graphic image (sign) of organic products; defines the basic requirements for the production of organic products; provides for voluntary confirmation of the conformity of its production".

Organic farming is already practiced in 187 countries around the world. The organic food market is one of the most dynamically developing ones in the world. From 2000 to 2019, it grew more than seven times (from 18 to 129 billion US dollars), showing its maximum growth in 2018-19 what was more than 16% per year. The number of people who constantly consume organic products in the world has also increased at least fivefold in 15 years, and amounted to about 700 million people. The leading consumers of organic products are the USA, EU countries and other developed countries, as well as China. In 2016 For the first time, European countries have recognized that the market for the consumption of organic products in the EU is growing faster than their production [1].

RESULTS AND DISCUSSION

The main inhibiting factor in the growth of organic agriculture in the world is the lack of suitable land resources, which gives great prospects for Russia, where more than 30 million hectares of fallow lands have not received agrochemicals for more than 3 years. Therefore, they may be suitable for introduction into circulation as organic ones with a short conversion period of one year. Currently, the area of "organic" lands in Russia is only 0.1% of all agricultural land, the share in the world market is only 0.17%. According to the statistics of the European Commission, in 2018 the EU imported a total of 3.3 million tons of organic agricultural products. The largest supplier of organic products in the EU is China. It accounts for 12.7% of imports or 415,243 tons of products. The share of imports of organic products from Russia was only 1% (34 thousand tons of organic products and raw materials, the 21st place among exporting countries). Agriculture is one of the branches of the modern Russian

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economy that can compete with other countries on the world market. There is a steady increase in demand in the organic market, and it far exceeds supply [2].

All the EU countries, the USA and other ones are ready to buy Russian products. The most important condition for long-term and mutually beneficial cooperation is compliance with strict organic standards. In organic crop production, such standards include agrotechnological charts, which include all the necessary measures for the biological system of plant protection from diseases, pests and weeds and are part of the transparency plan for production processes [3].

The regulatory framework for the production of organic products in the Russian Federation, except for Federal Law No. 280, is the Interstate standard GOST 33980-2016 “Organic products. Rules of production, processing, labeling and sale”, in which fertilizers and soil-improving substances are given in Appendix A, and plant protection products and agrochemicals allowed in organic production are given in Appendix B. In addition, there are two national standards: GOST R 56104-2014 “Organic food products. Terms and definitions”, and GOST R 57022-2016 “Organic Production. The procedure for voluntary certification of organic production”. Accreditation in the national body – Rosaccreditation – became an indispensable prerequisite. In addition, the recommendations set out in the food standard “Organic Food Products” of the Codex Alimentarius Commission and other international standards are taken into account. Nevertheless, there are regulatory gaps and unresolved problems in the practice of organic crop production in the Russian Federation.

The main method of protecting plants from pests and pathogens in the production of organic products is the biological method. In addition, the standards allow the use of certain pesticides of plant and microbial origin, minerals, certain acids, alcohols and other compounds. In order to control plant pests, it is recommended, first of all, to use entomophages [4]. In the Russian Federation, the situation is complicated by the fact that its own commercial production of entomophages is practically not developed; up to 80-90%, according to various estimates, of entomophages used in Russia are imported, the main supplier countries are Ukraine, the Netherlands, Israel, Belgium, Italy, England and others. The author of this article is to note that the entomophages themselves, and the products in which they are delivered to the country, pose a great phytosanitary risk. Entomophages may be carriers of the *Pepino mosaic virus* and other viral plant pathogens. Also, plant pests may be present in the products – the hosts of entomophages during production, in addition, the name of the entomophage may not correspond to the real organism present in the products. In this regard, in most countries entomophages are considered to be quarantined products of high phytosanitary risk and are subject to phytosanitary control. The same procedure is provided for by the International Plant Protection Convention and a number of international and regional standards [5]. Unfortunately, in the Russian Federation, entomophages were removed from phytosanitary control after 2010 and are used in agricultural production without taking into account the negative consequences [6].

It is known that the countries of Central Asia, especially Uzbekistan, have managed to maintain large volumes of production of biological plant protection products. This direction is actively supported by the State Plant Quarantine Inspectorate under the Cabinet of Ministers of the Republic of Uzbekistan. It seems advisable to combine the scientific potential and practical application of the results in this direction between the Russian Federation and other interested parties, including in the form of public-private partnership.

The Federal Law “On Plant Quarantine” (No. 206-FZ) does not provide for a special regime for the production of organic products. In this regard, when quarantine objects are detected in production or in organic products, the same measures are provided as when these objects are detected in any other branch of crop production. At the same time, synthetic pesticides are usually used to localize and eliminate foci or disinfect products, even a single treatment with which immediately deprives the products of organic status, and the company is forced to undergo a conversion period of at least 2 years before the status is resumed. It is important to remark that such a problem exists not only in the Russian Federation, so the corresponding wording was introduced in Paragraph 7.3. “Codex Alimentarius. Organically produced foods”: “The authenticity of the product should be maintained after import through to the consumer. If imports of organic products are not in conformity with the requirements of these guidelines due to treatment required by national regulations for quarantine purposes that is not in conformity with these guidelines they loose their organic status”. Such measures have a particularly negative impact on organizations that produce organic products for export, since they, in addition to being deprived of their status, have to bear costs due to non-fulfillment of contracts. Probably, for organic crop production it is necessary to develop special measures for localization and elimination of foci of quarantine objects and disinfection of products. Here, in addition to the use of biological methods of plant protection, it is possible to use a number of pesticides of plant and microbial origin, as well as other substances permitted in organic crop production. The situation is complicated by the fact that many of them, widely used in the world, are not registered in the Russian Federation and do not have standards of application approved in Russia. Also, in the Russian Federation, the biophysical or autocidal method, widely used in

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the USA and a number of other countries, is not used to combat quarantine pest species. This method is based on the resettlement of sterilized males of the same species into isolated pest foci. Using this method, the United States eliminated the Mediterranean fruit fly throughout the country.

In modern Russia, entomophages are mainly used in protected soil enterprises, primarily due to the high resistance of pests to pesticides. But almost all the greenhouse plants in Russia currently produce products on hydroponics, which, according to current legislation, excludes their participation in the production of organic. In the open ground, where organic production is currently developing, entomophages and other biological agents are much less used. Producers prefer to solve the problem of plant protection with proper agrotechnics and the selection of resistant varieties, compensating for the inevitable crop losses with a higher price for organic products. However, these methods are not always sufficient, even without force majeure. For example, the largest areas in the world – 36% of all sown areas with organic certification are allocated for wheat crops. In the production of organic wheat, only 2-3% of grains damaged by the bug harmful turtle – the main pest of grain crops in Russia, so that strong wheat loses the ability to improve the quality of flour with low baking properties. 3-5% of the grains damaged by the turtle make the products no longer suitable for baking bread. Methods of separating damaged grains and changing the baking technology have been developed to neutralize the enzymes secreted by the bug, but this significantly increases the cost of already not cheap products. It is much more technologically advanced to use egg-eating entomophages to combat the harmful turtle in the field. Methods of mass production and large-scale application of these entomophages are currently well developed. Methods of using entomophages to control the Colorado potato beetle in the production of "organic" potatoes have also been developed. In real organic crop production there is a so-called presumption of guilt, i.e. the manufacturer is obliged to prove that the agrotechnological charts of production provide for all situations, including all necessary measures for the biological protection of plants from diseases, pests and weeds, and they are supported by the availability or possibility of acquiring and using appropriate biological agents. At the same time, the detection of "reserve" synthetic pesticides during inspections is the basis for depriving an enterprise of the status of organic.

Conclusion

Opponents of organic agriculture call it a conservative and archaic way of production. This is far from true, except in cases where the archaic nature of production is an end in itself, for example, in the interests of agrotourism. Organic production, in order to be effective, must use the totality of accumulated knowledge about the interactions of organisms in bio- and agrocenoses and use modern diagnostic and control methods. The modern level of knowledge, in particular, allows to solve almost any problems of biological control of pests and pathogens in open and protected ground.

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Socio-Ecological and Economic Efficiency of Investment Subsidies in Agriculture of Kazakhstan

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Abstract. Development of agricultural commodity producers in Kazakhstan is limited, as the source of investment financing is mostly its own funds. And the low share of attracted funding sources suggests that active government intervention is needed. Lack of investment is fraught with problems. Among them, destruction of the material and technical base of enterprises, a reduction in production and a decrease in food, respectively, national security are possible. At the same time, investment subsidies for environmentally harmful industries may be ineffective. It is important to support environmentally efficient enterprises with subsidies that reduce unemployment in the regions. The purpose of the study is to propose and substantiate recommendations for increasing the socio-ecological and economic efficiency of investment subsidies in the agricultural sector of Kazakhstan. The article uses the methods of logical, comparative, economic and statistical analysis, as well as tabular methods of presenting information. The criteria for measuring the economic efficiency of investment subsidies cover both socio-economic (jobs, taxes) and environmental indicators (use of fertilizers, pesticides). The author discloses uneven distribution of investment subsidies over the years and a group of regions that have economic sense in attracting investment. The survey proposes a mechanism for the transition to an intensive path of agricultural development with elements of an innovation strategy. The paper also takes into account the modernization potential of the agricultural sector of Kazakhstan. The article shows the greatest socio-ecological and economic efficiency of investment subsidies in agriculture of crop regions (Pavlodar, Turkestan Oblasts). The ongoing investment projects improve the production base of the regions without causing significant damage to the environment. Kostanay and North Kazakhstan Oblasts also demonstrate quite high rates. In other regions, the efficiency of investment subsidies should be significantly improved.

INTRODUCTION

Implementation of an innovative strategy in agriculture in Kazakhstan seems to be very real based on the modernization potential of the industry, the main components of which are the natural resource component and the industry component. The natural resource component is characterized by large natural resources, not only already involved in the economic turnover, but also available for development in modern conditions. The sectoral component takes into account the structure of employment in the sector, the specifics of the economy, the geographical division of labour. The innovative model implies the technological development of agriculture, which will be able to provide an increase in profitability, profit and a positive social effect. And these signs will ambiguously contribute to the widespread development of investment activities in agriculture.

Strengthening of the agricultural export orientation determines its structural changes, which are often negative. The growing share of sub-sectors using natural resources contributes to the depletion of natural resources, especially soil. In the republic, the transition to resource-saving and clean technologies, moving them towards greening [1], is relevant. At the same time, opportunities for the ecological stability of the territory and rational use of natural resources are being reduced. Pastures in the modern state are hotbeds of desertification, but, despite this, they are used irrationally. Thus, the practical possibility of ecological balance of territories is limited [2].

The most logical way to use the funds received by entrepreneurs from subsidies would be to partially repay the principal debt on loans. This reduces not only the cost of lending, but also the base for calculating remuneration (interest), which will have a positive effect on the cost of production. Due to investment subsidies, investors will be

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less motivated to save on investment costs. If only the need for investment is not restrained by the need to pay off debt on loans [3]. But in this case, a positive effect can be noted, consisting in the fact that this will give them the opportunity not to save on technologies, to choose more expensive and, possibly, better quality equipment.

The result of investment subsidies can be discounted revenue streams for the period of the subsidized investment project or discounted tax flows. Although J. Svoboda et al. [4] showed that subsidies do not always increase income and investment in EU countries. S. Decramer and S. Vanormelingen [5] clarify here that this pattern is typical only for large farms. That is, subsidies help to raise incomes only among small farms. X.Z. Yin [6] demonstrates this relationship with smallholder R&D investments.

Unlike a commercial organization, the state is responsible for the implementation of its socially-oriented tasks. In this regard, when determining the ratio between costs and results of state support, it is necessary to take into account as results not only the achievement of the parameters specified in the state program, but also the accompanying social effects [7]. For example, this can be expressed as an increase in the number of jobs supported by investment projects in agribusiness [8, 9].

Materials and Methods

The socio-ecological and economic effect of development of investment subsidies can be determined by formula (1):

$$T_{Inv} = \frac{S\left[\omega\left(V-R\right)-S\right]+0.07A\cdot\Delta GRP+11NF+12s\sum_{i=1}^{n}K_i}{B+P_U Z_U+P_{Fert}H_{Fill}+P_{Pas}G_{Pas}+P_{Land}W_{Land}+\varphi X_{Spoilt}}, \quad (1)$$

where S is the amount of investment subsidies for agriculture (US$);

V is the value of gross agricultural production (US$);

B is the total cost of agricultural production (US$);

ω is the coefficient of elasticity reflecting the increase in the volume of agricultural products under the influence of an increase in the volume of subsidies by 1% (in unit shares);

R is the value of agricultural products attributable to the share of personal subsidiary plots (US$);

A is population size in the region, the level of food security of which is improving under the influence of increased consumption of local agricultural products (people);

ΔGRP is the increase in gross regional product *(GRP)* per capita for the estimated year of operation of the enterprise (US$ per capita);

0.07 is the projected increase in *GRP* in connection with the implementation of a social project (for each point of increase in *GRP*, respectively), according to the data of the Ministry of Economic Development of the Russian Federation [10];

11 is the maximum permissible period of stay in the status of unemployed registered with the employment service in accordance with the current Russian legislation on support of the unemployed (11 months);

N is the number of unemployed employed to work at the enterprise, in the investment project (people);

F is the average amount of unemployment benefits per month per person (US$);

12 is the number of months in a year during which the formula takes into account the receipts of social tax from hired workers;

s is social tax rate (in accordance with the current tax legislation) (in unit shares);

K_i is average monthly wages of hired workers (US$);

B is the total cost of agricultural production (US$);

$P_{U.}$ is cost rate for disposal or neutralization of agricultural waste (US$ per tonne);

$Z_{U.}$ is volume of generated agricultural waste (tonne);

$P_{Fert.}$ is average price for mineral fertilizers (US$ per tonne of active substance);

H_{Fill} is the amount of mineral fertilizers required to replenish nutrients in the soil after their consumption by agricultural crops (US$ per tonne of active substance). This indicator is calculated as the product of the area under crops of an agricultural crop and the reference rate for the absorption of nutrients from the soil in tonnes of active substance per hectare;

$P_{Pas.}$ is cost rate for restoration of pastures degraded by livestock grazing (US$ per hectare);

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$G_{Pas.}$ is area of pastures degraded by livestock grazing (hectares);

$P_{Land.}$ is the rate of costs for the reclamation of agricultural land, depleted and degraded due to agriculture (US$ per hectare);

$W_{Land.}$ is the area of agricultural land depleted and degraded by agriculture (hectares);

φ is the weighted average cadastral value of agricultural land completely withdrawn from economic circulation due to the irrational and destructive nature of agricultural activities (US$ per hectare);

X_{Spoilt} is the area of agricultural land completely withdrawn from economic circulation due to irrational and destructive agricultural activities (hectares).

The calculation of the coefficient of social, environmental and economic efficiency from the development of investment subsidies can be made according to the following formula:

$$E_{Inv} = \frac{T_{Inv}}{S} \quad (2)$$

The socio-ecological and economic effect calculated by formula (2) should be divided by the amount of allocated investment subsidies. If the resulting coefficient is higher than 1.5, then this fact characterizes a high return on subsidies. If it is below 1.0, then this indicates a low efficiency of subsidies. And if the coefficient is in the range of 1.0-1.5, it means a satisfactory level of return on the invested subsidies.

The ecological and economic effect of the development of the aggregate amount of subsidies in agriculture can be determined from formula (2), after removing all terms from the numerator of the fraction, with the exception of $S\left[\omega\left(V-R\right)-S\right]$, due to the fact that the social effect on the scale of the total amount of subsidies in agriculture is extremely difficult to calculate, therefore, it would be rational to limit itself to calculating only the ecological and economic effect.

Not every case of providing state support should be recognized as useful if we proceed from the position of the need to protect the environment. If, in connection with state support, an investment project in the field of agribusiness was implemented, which subsequently causes great damage to the environment, then the state support provided in this case should be recognized as harmful from an environmental point of view.

Results

In the investment activity of agriculture, there is a lag behind other sectors of the real sector. This can be easily seen from the share of investments in agriculture in their total volume in the country's economy. The average value of the share of agricultural investments over the past ten years was 2.36%, which is low compared to other sectors of the economy. The dynamics of investment subsidies is highly uneven over the years (Table 1).

TABLE 1. Dynamics of investment subsidies for agricultural producers in Kazakhstan in 2015-2020

Indicator	2015	2016	2017	2018	2019	2020
The amount of subsidies paid in 2008 prices, US$ millions	17.4	43.6	120.7	67.8	126.9	119.0
Growth rate, %		150.6	176.8	–43.8	87.2	–6.3

Source: calculated by the author based on the materials [11-12].

As a result of application of formulas (1) and (2), regional coefficients of the socio-ecological-economic efficiency of investment subsidies in agriculture were obtained. The largest indicator of the coefficient of socio-ecological and economic efficiency in 2020 was noted in the Turkestan and Pavlodar Oblasts (over 1.6), where crop production is the predominant type of agriculture. Lagging positions were taken by Aktobe, Kyzylorda and East Kazakhstan Oblasts (0.6-0.7). Grain-sowing regions stand out as average indicators: North Kazakhstan, Kostanay and Akmola Oblasts (within 0.9-1.1).

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Discussion

In contrast to our position, A. Korzhenevyc and J. Bröcker [13] argue that investment subsidies are harmful from the point of view of public welfare. In addition, they can distort market conditions and are therefore subject to WTO accounting. One of the possible options for solving the problems of investment activity in agriculture is the choice of the correct investment policy, the key task of which is the formation of an effective mechanism. The role of the state in providing support to agriculture in attracting financing is very high. However, within the framework of the WTO rules, direct government intervention in agriculture is limited, in modern conditions it is possible to use measures that will attract private capital investments in the agricultural sector and will not be restricted by the WTO. The scaling up of investment subsidies should be guided by WTO rules and their impact on markets. We agree with the view of S. Wang et al. [14], claiming the positive impact of investment subsidies on the innovation activity of enterprises.

Conclusion

The low rates of renewal of the park of machines and equipment form a large technical and technological gap between the agriculture of Kazakhstan and developed countries. One of the main problems of investment activity in the agriculture of Kazakhstan is that investments are used ineffectively due to the extensive development of the agro-industrial complex. By solving this problem, the active participation of the state in scientific and innovative activities and the creation by the state of conditions under which the scientific and innovative component will be the main tool for gaining competitive advantages by agricultural producers seems to be.

Investment subsidies should be aimed at innovative renewal of the agro-industrial complex of Kazakhstan. This is an integrated approach differentiated by region, taking into account the specialization of regions, their soil and climatic conditions. Here we are not talking about the acquisition of individual highly productive units, but about the introduction of machine systems that will ensure the automation and mechanization of production processes of the entire complex. Complex technological solutions mean the intensification of all components of the agricultural production process. Intensive varieties and hybrids of agricultural crops, animal breeds, mineral and organic fertilizers, protection of crops and animals from pests and diseases, etc. will be used.

In an integrated approach, the possibilities of using investment subsidies get a special role. In the long term, this contributes to increasing the stability and efficiency of agricultural production, expanding the energy base of the industry, strengthening its reproductive autonomy, increasing the competitive potential of agricultural enterprises in the energy market.

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Competitiveness of Non-Urbanized Territories as a Basis for Their Sustainability

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Abstract. It is difficult to imagine the development of a single country without the proper development of its regions and municipalities, which are part of both the national and global economies. Geographical, economic, social, historical, and cultural features lead to quite a large differentiation in the development of territories, especially non-urbanized, due to the vastness and polyethnicity of the Russian Federation. It follows that each territory must develop its unique competitive advantages. The essential need to consider territories as participants in social and economic relations in a competitive environment comes to the fore. The situation is particularly difficult in non-urbanized territories characterized by the problems of falling density and population aging, its standard of living and life quality, deterioration of economic activity, the state of industrial and social infrastructure. All this deprives these territories of stability, anti-fragility. This begs the question: what can enable nonurbanized entities to survive in competition for human and economic resources? This study aims to determine the algorithm for the formation and mechanism of implementation of the competitive advantages of these locations. The article proposes an algorithm for a comparative analysis of competing territories to identify the advantages of a particular territory and proposed a system of indicators (by blocks) to conduct this analysis. A comprehensive mechanism for implementing competitive advantages is offered in the example of a particular municipality.

INTRODUCTION

Socio-economic conditions are necessary for the formation of sustainable production and consumption patterns. In one way or another, all of them are related to the quality of life and profitability of doing business in a particular area. At the same time, all locations, both state and in-country, compete for all kinds of resources. The so-called non-urbanized territories are in the weakest position.

Meanwhile, the development of non-urbanized territories (in Russia, the administrative-territorial approach is more commonly used – municipalities) in a competitive environment does not receive sufficient attention from state authorities, local government, and civil society. As a rule, development is considered to a limited range of territories with a special innovative status, such as science cities [1]. Such an approach leads to an increase in disproportions in developing the remaining territories, reducing the life level and quality [2]. This causes a drop in agricultural production (sustainable production) and reduces demand due to decreased purchasing power (sustainable consumption).

Competition, if it is not competition for privileges, building an exclusive relationship with the state is the engine of economic development. The core of competition, and economic development, is the process of innovation, which results in new products, new technologies, resources and markets, and ways of organizing production [3, 4]. The presence of rational competition is a factor that provides economic and technological development of the state and acts as a mechanism to increase the investment attractiveness and increase the inflow of foreign investment into the country [5].

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Competition is the most important source of social welfare growth and, potentially, a means of solving a variety of social problems. Not only material issues – low real income per capita, but also the problem of satisfying the human need for self-realization, based on the possibility of choosing from the many available alternatives of activity [6].

It is not uncommon for territories to face a complete lack of interest in their products. This requires incentive marketing, which is effective if the territory manages to clearly diagnose its competitive advantages and intensively promotes them [7]. For example, we can talk about almost any sparsely populated and (or) remote territory, which is commonly classified as non-urbanized.

According to the European concept of "smart specialization", the basis for forming a strategy for the development of territories is competitive advantages. To do this, an analysis should be carried out on the parameters of each subject's advantages: natural and geographical conditions, industry, transport links, demographics and the level of the working capacity of the population, and infrastructure. The result of this analysis will be a list of subjects with competitive advantages, which can become the center of the sustainable development strategy of the country, region, and municipality. The purpose of this strategy is to stimulate and enhance the territory's economic development [8].

It is worth noting that the strategic development of territories is associated with the globalization of the world economy [9, 10]. The territory cannot be competitive in all areas of the economy. It is required to pay attention to those sectors in which the territory has a clear advantage. The municipality needs to specialize in certain economic functions and ensure their complementarity. In this regard, this study aims to improve the methodological approaches to determining the competitive advantages of non-urbanized territories.

Materials and methods

Ust-Tarksky District of Novosibirsk region, as one of the smallest and most remote from the Novosibirsk agglomeration, was chosen as the object of the study. Methods of comparative analysis, rating, SW-analysis, simulation modeling were used to identify the competitive advantages of the municipal district as prerequisites for its competitiveness and sustainability of development.

Results

To identify the competitive advantages of a location, it is necessary to carry out its comparative analysis with competing territories. The authors propose the following algorithm:

1. Criteria selection for determining the territory's external and internal competitive advantages, bringing them into a system of indicators.
2. Sampling numerical values by relevant indicators for the sought municipal district and territories – direct competitors.
3. Determination of the place (rank) of the district for each of the indicators.
4. Identification of strong – first place and weak sides of the territory – the last place in the ranking list of criteria (indicators).
5. Determination of the current positioning of the territory.
6. Creating the municipality's growth points and development directions based on the competitive analysis.

A comparison was made with the neighboring municipal districts of the Novosibirsk region – Vengerovsky and Tatarsky to identify the strengths and weaknesses of Ust-Tarksky District. Table 1 presents indicators of comparing districts by social block [11-13].

TABLE 1. Comparative indicators of competing territories in the social block (population and labor resources) for 2020

Indicator name	Ust-Tarksky District	Vengerovsky District	Tatarsky District
Permanent population, people	12850	18047	37259
People employed in the economy, people	4380	6612	19973
Average per capita monetary income of the population, RUB	9997	16867	24350

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Average pension size, RUB	12056	12435	14042.35
Rating score, point	1	2	3

Note: 3 points – maximum, 1 point – minimum

Table 2 presents indicators comparing competing territories in industry and agriculture [11-13].

TABLE 2. Comparative indicators of economic potential for 2020.

Indicator name	Ust-Tarksky District	Vengerovsky District	Tatarsky District
Volume of own-produced goods shipped, work and services performed, RUB mln.	187.2	736.5	4000
Industrial production index, %	101.2	105.1	100.6
Volume of agricultural production, RUB mln.	1498	2885.6	4928
Rating score, point	1	2	3

Note: 3 points – maximum, 1 point – minimum

Thus, the analysis allowed us to determine that Ust-Tarksky District ranks third. Table 3 shows the comparison indicators of the territories-competitors on the infrastructure block [11-13].

TABLE 3. Comparative indicators for the infrastructure block for 2020

Indicator name	Ust-Tarksky District	Vengerovsky District	Tatarsky District
Total length of roads in the district, km	497	490.8	1004.9
- of them with a hard coating, %	44	64	85
Railway lines on the territory of the district	missing	missing	mainline of the West Siberian Railway
Logistics facilities in the area	present in a small amount	missing	present
Rating score, point	2	1	3

Note: 3 points – maximum, 1 point – minimum

Ust-Tarksky District is in second place in terms of infrastructure indicators, behind Tatarsky District.

The results of the comparative analysis are illustrated using the competitiveness polygon (Fig. 1).

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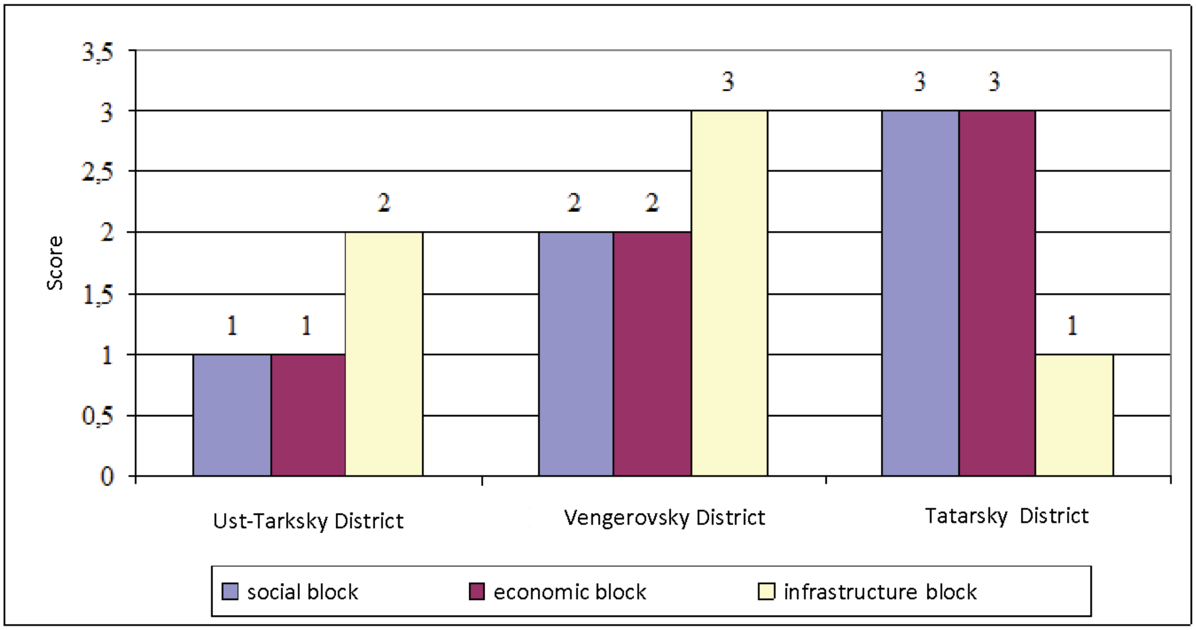

FIGURE 1. Competitiveness assessment polygon

The analysis showed that Ust-Tarksky District, in terms of competitive advantages, is inferior to the neighboring districts – Vengerovsky and Tatarsky. However, it has unique competitive advantages. Table 4 shows the peculiarities of functioning of competitive advantages realization of Ust-Tarksky District.

TABLE 4. Features of implementation of competitive advantages of Ust-Tarksky District. Compiled by the authors

Competitive advantages	Positive features	Negative features
Economic and geographical position	The district has a relatively favorable transport-economic and geographical position Special border position: the western border of the district is the border with the Omsk region	Logistics facilities in the area are present in an insignificant amount (e.g., storage facilities) and are not systematically linked
Tourism potential	The district has conditions for the development of recreational and tourist potential (e.g., there is a biological reserve Ust-Tarksky, 82 thousand hectares in the district, a lake Uguy with decay ooze)	There is only one hotel in the area – "Pearl of Siberia", the tourist infrastructure is underdeveloped.
Natural resources	There are proven mineral resources: reserves of brick clay to meet the district's own needs (raw materials for brick production) 6.5% of the area is occupied by forest-steppe and birch forests; the main soils are ordinary chernozem The Om River, one of the largest rivers in the region, flows through the area; the lakes are located mainly in the lowlands, they are inhabited by commercial fish species: crucian carp, perch, pike; the area is	Increase in the volume of harmful emissions into the atmosphere Poor drinking water quality

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	attractive for recreational fishing and hunting. Agricultural land can be expanded with 11.5 thousand hectares of reserve land.	
Housing stock	Availability of reserve areas suitable for housing construction	Availability of the total floor area of the housing stock is below the regional average
Industrial potential	Availability of existing enterprises of the food industry Availability of enterprises making products for export. Reserve of industrial sites for production location	Agricultural products are mainly exported outside the district for further processing

Discussion

The analysis showed that Ust-Tarksky District has many weaknesses that affect its competitiveness and sustainability. However, there are also significant competitive advantages – superiority in economic, social, cultural, and other areas of development that make it, in comparison to other non-urbanized areas, more attractive to residents, highly skilled workers, investors, and tourists.

Municipal authorities should focus on the following priorities:

1. determine directions for the development of the region's attractiveness to its consumers,
2. assess resources for the development of competitive advantages of the region and compare them with the resources of competing regions;
3. assess obstacles to the development of the region's competitive advantages and relate them to the available resources.

Following the program-targeted budget planning method in Ust-Tarksky, the District Development Strategy is being implemented. However, it does not consider the competitive position of the territory and needs to be updated.

Based on the above, we have developed and proposed a model of an integrated mechanism for implementing competitive advantages of Ust-Tarksky District (Fig. 2.).

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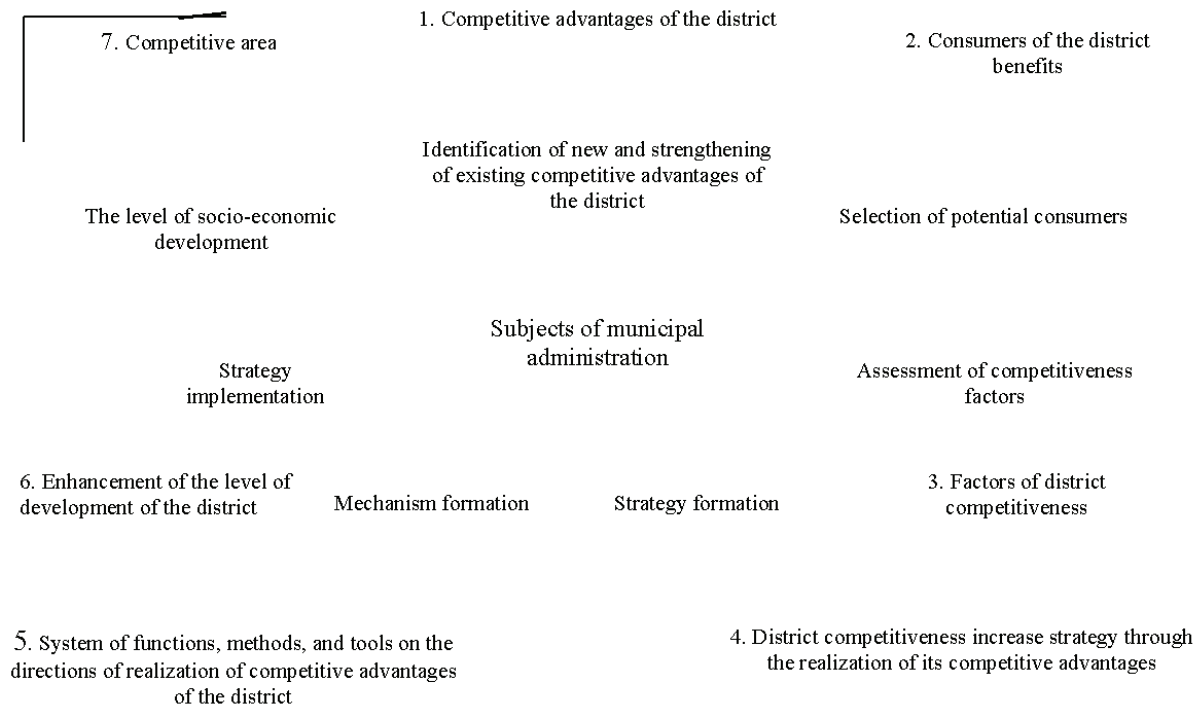

FIGURE 2. Comprehensive mechanism for the implementation of competitive advantages of Ust-Tarksky District

At the first stage, the existing competitive advantages of Ust-Tarksky District are identified and evaluated to further strengthen and implement them. We identified competitive advantages, which are presented in Table 4.

The second stage is to identify potential consumers of the region's competitive advantages.

At the third stage, it is important to determine what factors contribute to competitiveness, including abandoning unpromising competition in areas with little chance of attracting or retaining customers. For each consumer, there is a set of district competitiveness factors. Each of these factors shows the presence of certain properties that are particularly valued by the consumer when deciding whether to settle, locate production facilities, move cargo or visit this place by tourists. This is the reason for the inclusion of the third stage in the model of the mechanism of realization of competitive advantages.

The result of the first three stages is the development of a strategy to improve the competitiveness of the area by implementing its competitive advantages in the fourth. At this stage, goals, and objectives for developing the competitiveness of the non-urbanized territory are defined.

At the next stage, the methodology and tools of the mechanism of realization of competitive advantages of the location are formed. At the same time, municipal authorities must ensure the implementation of the necessary and most innovative organizational methods that act as catalysts, accelerating the development and adding value to the area.

The implementation of the district competitiveness improvement strategy, through the implementation of the proposed comprehensive mechanism for the realization of its competitive advantages, is expected to intensify the increase in the level of socio-economic development of the territory [14].

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CONCLUSION

In addition to the proposed comprehensive mechanism for the implementation of competitive advantages of Ust-Tarksky District, the implementation of the following elements is recommended:

1. Introduction of strategically oriented municipal management, updating the municipality's existing planning and forecasting documents.
2. Informatization and implementation of the principles of e-government, which can provide the non-urbanized territory with a serious competitive advantage.
3. Active positioning of Ust-Tarksky district. The method of active positioning is fairly new. Still, it is based on taking into account such widespread and developing phenomena as competition and neighborhood competition, on the one hand, and partnership and cooperation of different types of territorial formations, on the other.

The introduction into practice of the proposed recommendations, mastering the mechanisms and methods of strengthening the competitiveness of Ust-Tarksky District becomes objectively necessary and can serve to activate the internal potentials of the district and an additional factor in ensuring the sustainability of strategically oriented municipal development.

Competition is an integral part of the market economic system. The beneficial effect of competition on sustainable development and the need to maintain a high level of competition in almost all markets is beyond doubt [15]. We believe that competitiveness is the most appropriate means of regulating basic socio-economic processes, distributing material goods, satisfying the interests of consumers of resources of non-urbanized territories.

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Approbation of Methods for Detecting Highly Dangerous Phytoplasmas from the Apple Proliferation Group

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Abstract. Phytoplasma diseases of pome crops are of economic importance, both in foreign countries and on the territory of the Russian Federation. One of the harmful phytopathogens are phytoplasmas *Candidatus* Phytoplasma mali (causative agent of apple proliferation) and *Candidatus* Phytoplasma pyri (causative agent of pear decline). The spread of phytoplasmas is carried out with the help of psyllas and leafhoppers, infected planting material, and parasitic plants. Symptoms of infection with these phytopathogens are branching shoots, small leaves with enlarged stipules, yellow or orange shade of leaves, necrosis. Phytoplasma infection may be in a latent state without symptoms. Diagnostics of phytoplasmas using molecular genetic diagnostic methods will allow the use of measures to limit the spread of harmful microorganisms. This paper presents part of the research on the diagnostics of pathogens of apple proliferation and pear decline, according of Diagnostic Protocols for detection harmful organisms. Universal primers and a probe for detection of *Candidatus* Phytoplasma mali and *Candidatus* Phytoplasma pyri using Russian reagents have been tested. The research was conducted on the basis of All-Russian Plant Quarantine Center (FSBI “VNIIKR”) together with colleagues from LLC Syntol and RUDN University.

INTRODUCTION

Gardening is an integral branch of agriculture in the Russian Federation. In the spring of 2021, the Ministry of Agriculture approved the subprogram “Development of nursery gardens and horticulture” within the framework of the Federal Scientific and Technical Program for Agriculture Development for 2017-2025 (Decree of the Government of the Russian Federation No. 996 dated August 25, 2017). The federal program is aimed at ensuring environmentally friendly agriculture, planting fruit plantations, the use of plant material protection products, the creation of high-quality agricultural products, planting material. One of the main objectives of the program is the development of modern diagnostic tools for pathogens of plant material. According to the annual report “The market of fruits of seed crops (apples, pears, quince) in Russia [1]. The current situation and forecast for 2021-2025” of the analytical company Alto Consulting Group (ACG), in the Russian Federation for 2020, the gross harvest of seed crops (apple, pear, quince, etc.) amounted to 23,416.5 TMT, which is 7.4% more than the production volume of the previous year.

Phytosanitary risk for horticulture in Russia is represented by phytoplasmoses of fruit crops. Pathogens of apple proliferation (*Ca.* P. mali) and pear decline (*Ca.* P. pyri) lead to a decrease in the yield of seed crops in the zone of their cultivation to 80-95%, therefore, these types of microorganisms are included in the List of Quarantine Pests of

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the Eurasian Economic Union (II. Quarantine pests of limited distribution on the territory of the Eurasian Economic Union) [2-4].

Microorganisms *Ca.* P. mali and *Ca.* P. pyri are representatives of the apple proliferation group, 16SrX [5, 6]. The main host plants are the domestic apple tree *Malus domestica* Borkh. and the common pear *Pyrus communis* L., however, there is evidence of infection of other types of crops: common quince *Cydonia oblonga* Mill., Chinese plum *Prunus salicina* Lindl., common peach *P. persica* (L.) Batsch, common hazel *Coryllus avellana* (L.) H. Karst., grape vine *Vitis vinifera* L. et al. [7, 8]. Apple and pear varieties have different susceptibility to the disease of apple proliferation and pear decline. During the growing season, phytoplasma spreads through the phloem of the aboveground parts of plants, and during winter migrates through sieve tubes into the root system. For the causative agent of apple proliferation, the phenomenon of "recovery" is known, when a high titer of phytoplasma remains in the infected plant, but the obvious symptoms of the disease disappear. It is assumed that this phenomenon is associated with antagonistic interaction between avirulent and virulent strains of phytoplasma. The optimal temperature for the development of phytoplasmas is +21-25 °C [8-10].

These types of phytoplasmas are widely distributed in Europe (more than 27 countries), Asia, and America [11]. In the National Report on the quarantine phytosanitary condition of the territory of the Russian Federation in 2020, it was noted that for the first time pathogens of apple proliferation and pear depletion were detected in the country [12].

To prevent the further spread of phytopathogenic microorganisms, it is necessary to search and optimize molecular genetic methods for detection of harmful organisms. The Diagnostic Protocols of the European and Mediterranean Plant Protection Organization (EPPO PM 7/133 (1) and EPPO PM 7/62 (3)) propose universal primers and probes for the diagnostics of phytoplasmas [13, 14].

The work objective is to study and optimize the diagnostics of pathogens of phytoplasmosis of the apple proliferation group using molecular genetic methods with reagents of domestic origin.

Materials and Methods

6 types of phytoplasmas were used in the research work: from the group 16SrX – *Ca*. P. mali (causative agent of apple proliferation); *Ca*. P. pyri (pathogen of pear decline); *Ca*. P. prunorum (causative agent of European stone fruit yellows); from group 16SrXII – *Ca*. P. convolvuli (the causative agent of bindweed yellows); *Ca*. P. solani (causative agent of bois noir, stolbur); from the group 16SrV – *Ca*. P. rubi (causative agent of rubus stunt).

To determine the analytical sensitivity, a dilution series of samples (from 10^{-1} to 10^{-7}) containing DNA of *Ca* P. mali and *Ca*. P. pyri phytoplasmas was used in two repetitions. To check the PCR reaction, internal positive control (IPC) synthesized by the Russian company LLC Syntol (Syntol) was used.

The DNA of phytoplasmas from plant material was isolated using a set of reagents CytoSorb (Syntol, Russia) and according to the Doyle & Doyle method (EPPO PM 7/133(1)) optimized by specialists of the FSBI VNIIKR. Preliminary preparation of samples for isolation by a commercial set of reagents was carried out as follows: 500 mg of plant material was homogenized in a ceramic mortar in 3 ml of extraction buffer. 1 ml of a homogeneous sample was transferred into 1.5 ml Eppendorf-type test tubes. Further DNA isolation was carried out according to the instructions for the kit. For the extraction of microbial DNA, 1 mg of plant tissue was selected according to the Doyle&Doyle method. The studied material was crushed in 30 ml of a grinding buffer in a ceramic mortar. The resulting homogenate was transferred to macrocentrifuge tubes with a volume of 50 ml, centrifuged with cooling at 5,700 rpm for 5 minutes at +4 °C. The resulting supernatant was transferred to new macro tubes and centrifuged again at 9,500 rpm for 25 minutes at +4 °C. The resulting precipitate contained DNA of phytoplasmas. Further DNA isolation was carried out according to the method. Extraction of each sample was carried out twice.

For real-time PCR, a probe and primers developed by Christensen et al. (2013) based on the alignment of the 16S rDNA gene in some strains of phytoplasmas, bacteria, mycoplasmas were used. For the convenience of using primers and the probe, the authors of the study gave the names Uni2-F, Uni2-R, and Uni2-probe respectively. We used a ready-made unstained mix for PCR 5X qPCRmix-HS (Evrogen, Russia). Reaction mixture composition for amplification of one test sample is as follows (total volume is 25 µl): –2 µl. The amplification program with a pair of UNI2-F/UNI2-R primers and a UNI2-probe consisted of the following stages: 1) 95 °C – 5 min; 2) 45 cycles: 95 °C – 15 sec, 60 °C – 1 min. Amplification was carried out on a DTprime device (DNK-Technology, Russia).

Universal primers P1/P7 (Deng&Hiruki, 1991; Schneider et al., 1995) were used for the first stage for nested PCR. This pair of primers amplifies the entire length of 16S gene, the intergenic spacer of 16S-23S gene, as well as a small part of 23S rRNA (about 1850 bps). Universal fU5/rU3 primers (Lorenz et al., 1995) were used as the

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second stage of nested PCR, the amplification product is about 870 bps. We used a ready-stained mix of 5X Mas ^{DD} Taq Mix-2025 (Dialat Ltd, Russia). Reaction mixture composition per sample for the first stage of nested PCR with a pair of primers P1/P7 and the second stage with primers fU5/rU3 is as follows (total volume is 25 µl): deionized water – 14.5 µl, mix 5X Mas ^{DD} Taq Mix-2025 – 5 µl, P1 (fU5 for the second stage) – 1.5 µl, P7 (rU3 for the second stage) – 1.5 µl, total DNA (amplification product of the first stage) – 2.5 µl. After the first stage of nested PCR, amplicons were diluted in deionized water (1:30 respectively). For the second stage, the diluted amplicon (the amplification product of stage 1) was added to the reaction mixture for the second stage of nested PCR.

Amplification program with P1/P7 primers: 1) 95 °C – 5 min; 2) repeated reaction for 35 cycles: 94 °C – 1 min, 52 °C– 2 min, 72 °C – 2 min 30 sec; 3) 72 °– 5 min. Amplification program with fU5/rU3 primers: 1) 95 °C – 5 min; 2) repeated reaction for 35 cycles: 94 °C – 30 sec, 57 °C – 30 sec, 72 °C – 50 sec; 3) 72 °C – 5 min. The SimpliAmp thermal cycler (Applied Biosystems, USA) was used. Horizontal electrophoresis in 1.5% agarose gel was used to visualize the results. DNA LADDER SM0323 molecular weight marker with a range of 100-3000 bps (Thermo Fisher Scientific, USA) was used as well. Electropherograms were obtained using the Bio-Rad gel-imaging system (USA).

Results

When using a pair of UNI2-F/UNI2-R primers and a UNI2-probe, analytical sensitivity results were obtained using the DNA of pathogens of apple proliferation and pear depletion, presented in Table 1.

TABLE 1. Results of analytical sensitivity of UNI2-F/UNI2-R primers and UNI2-probe using DNA of phytoplasmas *Ca*. P. mali and *Ca*. P. pyri

Sample	Cq, Rox Average value	Cq, Hex Average value	Sample	Cq, Rox Average value	Cq, Hex Average value
Ca. P. mali (in.)	20.4		*Ca*. P. pyri (in.)	28.0	29.7
Ca. P. mali (in.)	20.3		*Ca*. P. pyri (in.)	28.1	29.6
Ca. P. mali (-1)	24.0		*Ca*. P. pyri (-1)	30.3	31.2
Ca. P. mali (-1)	24.0		*Ca*. P. pyri (-1)	30.3	31.2
Ca. P. mali (-2)	27.9	30.1	*Ca*. P. pyri (-2)	33.6	32.6
Ca. P. mali (-2)	27.8	30.3	*Ca*. P. pyri (-2)	33.7	32.5
Ca. P. mali (-3)	31.0	31.6	*Ca*. P. pyri (-3)		33.2
Ca. P. mali (-3)	30.8	31.3	*Ca*. P. pyri (-3)	36.6	32.9
Ca. P. mali (-4)	33.6	32.5	*Ca*. P. pyri (-4)	38.6	32.9
Ca. P. mali (-4)	34.6	32.6	*Ca*. P. pyri (-4)		33.0
Ca. P. mali (-5)	36.6	32.9	*Ca*. P. pyri (-5)		32.9
Ca. P. mali (-5)	35.4	32.9	*Ca*. P. pyri (-5)		33.1
Ca. P. mali (-6)	36.4	32.3	*Ca*. P. pyri (-6)		32.9
Ca. P. mali (-6)	36.2	32.9	*Ca*. P. pyri (-6)		32.9
Ca. P. mali (-7)		33.0	*Ca*. P. pyri (-7)		32.7
Ca. P. mali (-7)	38.4	32.7	*Ca*. P. pyri (-7)		33.0
K- (H_2O)		32.7	K- (H_2O)		32.8

Source: compiled by the authors

According to the data obtained from Table 1, it can be seen that when PCR is set up with a pair of UNI2-F/UNI2-R primers and a UNI2-probe, the stable reaction takes place when the *Ca*. P. mali sample is diluted from 10^{-1} to 10^{-6}, *Ca*. P. pyri sample — up to 10^{-2} (Cq, Rox — specifics). The results on the Hex channel (IPC) show the absence of a false negative result, however, when the initial *Ca*. P. mali sample is introduced and when it is diluted 10 times, the reaction is inhibited, which may be due to the presence of inhibitors in the mixture.

Therefore, it can be concluded that the amount of the initial matrix of the sample affects the degree of detection of the phytopathogen during diagnostics.

The results on the analytical specificity of UNI2-F/UNI2-R primers and UNI2-probe using 6 types of phytoplasmas are presented in Table 2.

The results obtained from Table 2 show that a pair of UNI2-F/UNI2-R primers and a UNI2-probe probe revealed not only the studied types of *Ca*. P. mali and *Ca*. P. pyri phytoplasmas, but also other types of phytoplasmas from different groups, which indicates the versatility of this type of research.

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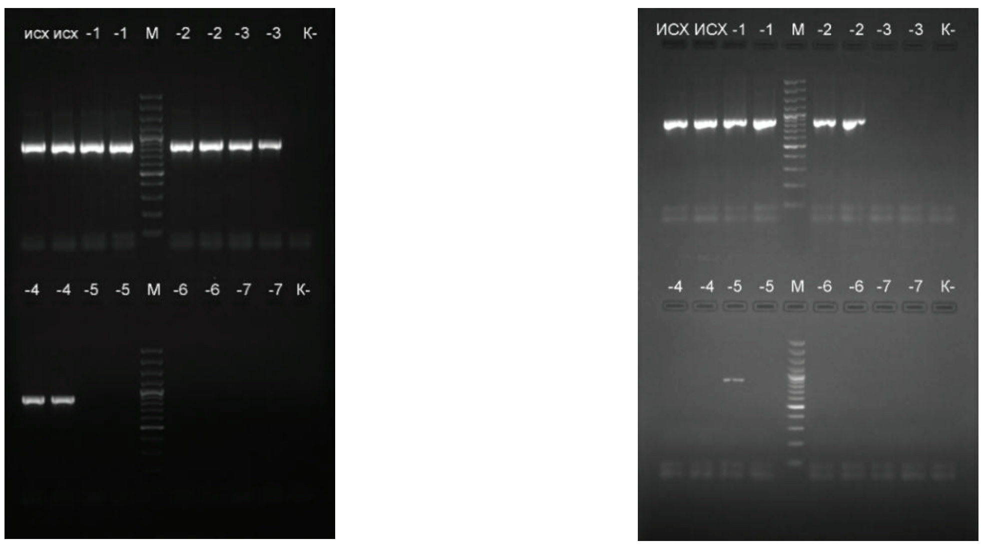
TABLE 2. Results of analytical specificity of UNI2-F/UNI2-R primers and UNI2-probe using 6 types of phytoplasmas

Sample	Cq, Rox Average value	Cq, Hex Average value
Ca. P. mali	24.0	32.4
Ca. P. pyri	20.6	32.6
Ca. P. prunorum	27.4	31.2
Ca. P. convolvuli	15.8	32.7
Ca. P. solani	26.4	32.6
Ca. P. rubi	21.4	31.9
K- (H_2O)		32.1

Source: compiled by the authors

In the study of target objects by nested PCR using pairs of primers P1/P7 and fU5/rU3, the following results on the analytical sensitivity of primers were obtained, presented in Figures 1 and 2.



a) *Ca*. P. mali (fU5/rU3, 870 bps) b) *Ca*. P. pyri (fU5/rU3, 870 bps)

FIGURE 1. Electrophoregram of the second stage of nested PCR with a pair of fU5/rU3 primers. Note: a, b – in. – initial concentration of the test sample; from -1 up to -7 – a series of dilutions of the sample from 10^{-1} to 10^{-7}; K- – negative control (H_2O). M – molecular weight marker 100-3000 bps

As can be seen from Figure 1a, the reaction with DNA of *Ca*. P. mali is stable when the sample is diluted 10,000 times. The reaction is not stable since the dilution of the sample by 100,000 times. The presented data from Figure 1b show the reaction using DNA of *Ca*. P. pyri is stable when the sample is diluted 100 times. When the sample was diluted 1000 times or more, the reaction was unstable and the target organism was not detected. However, when the sample was diluted 100,000 times, single pieces of the target organism were noted. Clear specific amplification products were obtained on electropherograms.

The results obtained on the analytical specificity of primers P1/P7 and fU5/rU3 during nested PCR using 5 types of phytoplasmas are shown in Figure 2.

The results presented in Figure 2 show that primers P1/P7 and fU5/rU3, developed on a part of the 16S rRNA gene, are able to identify not only target organisms of apple proliferation group, but also other types of phytoplasmas. Clear selective amplification products equal to about 870 bps were obtained on electropherograms.

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Discussion

Based on the obtained research results, the following conclusions can be drawn. The UNI2-probe and the primers UNI2-F/UNI2-R (Christensen et al., 2013), P1/P7 (Deng&Hiruki, 1991; Schneider et al., 1995), and fU5/rU3 (Lorenz et al., 1995) used for PCR show the versatility of diagnostics for different types of phytoplasmas. Results of analytical sensitivity of primers and probe for the studied types of phytoplasmas *Ca*. P. mali and *Ca*. P. pyri, show a different degree of detection of pathogens in seed crops. For the pathogen of apple proliferation, detection is clearly traced with UNI2-F/UNI2-R primers and UNI2-probe when diluting the pathogen DNA by 1,000,000 times, with pairs of primers P1/P7 and fU5/rU3 — by 10,000 times. Accordingly, for the causative agent of pear depletion – by 100 times. It is assumed that this is due to the fact that species *Ca*. P. mali and *Ca*. P. pyri have differences in the sequences of one gene of 1.0-1.1% [15].

According to the results of primers and the probe testing, it was found that the UNI2-probe and primers UNI2-F/UNI2-R (Christensen et al., 2013), P1/P7 (Deng&Hiruki, 1991; Schneider et al., 1995), fU5/rU3 (Lorenz et al., 1995) are suitable for screening samples of quarantined products. However, all obtained amplification products are subject to species identification analysis using the sequencing method.

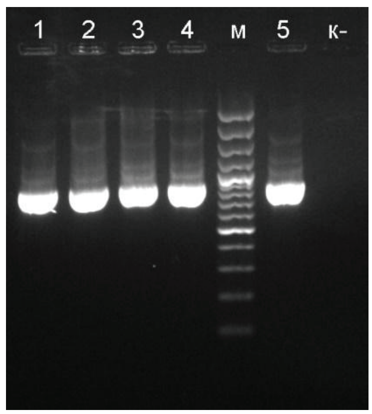

FIGURE 2. Electrophoregram of the second stage of nested PCR with a pair of fU5/rU3 primers. Note: 1 – *Ca*. P. mali; 2 – *Ca*. P. pyri; 3 – *Ca*. P. prunorum; 4 – *Ca*. P. rubi; 5 – *Ca*. P. convolvuli; K- – negative control (H_2O). M – molecular weight marker 100-3000 bps

Conclusion

The obtained results are part of studies on the diagnostics of phytopathogens *Ca*. P. mali and *Ca*. P. pyri. By having carried out a universal diagnostics of phytoplasmas, it is necessary to continue research on species identification of pathogens causing apple proliferation and pear depletion.

Positive results were obtained on the use of the UNI2-probe and UNI2-F/UNI2-R primers (Christensen et al., 2013), P1/P7 (Deng&Hiruki, 1991; Schneider et al., 1995), fU5/rU3 (Lorenz et al., 1995) with reagents from domestic manufacturers. The approbation of various primers has shown the opportunity of their use for the diagnostics of harmful organisms.

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Aspects of the Phytosanitary Biology of Three Highly Dangerous Species of Hemipterans (Insecta: Hemiptera)

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Abstract. Issues of biological and food security of the Russian Federation are associated with the invasive processes of pests. Plant quarantine plays an essential role in this direction, in which accurate and operational diagnostics are ensured. An essential aspect of timely detection of alien pests is predicting their potential spread. Reliable prediction of zones of the possible species acclimatization combined with data on possible pathways of distribution ensures early detection of the species in case of its introduction. In the present work, studies were conducted in the context of a phytosanitary diagnosis of three species of hemipterans (Insecta: Hemiptera) that pose a phytosanitary risk for the Russian Federation territory: *Chionaspis pinifoliae* (Fitch, 1856), *Lycorma delicatula* (White, 1845), *Megacopta cribraria* (Fabricius, 1798). Various preparation and microscopy methods were used to study the diagnostic signs of the above pest species and computer methods of habitat modeling. As a result of this research, approaches to morphometric studies of diagnostic signs of pygidium of the *Chionaspis pinifoliae*-*heterophyllae* complex for differentiation of *Chionaspis pinifoliae* and for the formalization of the measurement procedure, which is essential for the practice of phytosanitary laboratories; it is shown that *Lycorma delicatula* is well-differentiated from other species of the genus, while identification at the level of supraspecific taxa seems complicated and requires appropriate research; the results of modeling the *Megacopta cribraria* area for Russia showed a high probability of its distribution within the south of Krasnodar Krai and the Republic of Adygea, which should be considered when conducting surveys to identify this pest in case of its possible infestation. Reference specimens of the following species were isolated: *Chionaspis pinifoliae* deposited in the Zoological Institute of the Russian Academy of Sciences (St. Petersburg) and *Lycorma delicatula* from the collection of the Zoomuseum of Moscow State University (Moscow). The latter is of practical importance for developing and applying diagnostic protocols in plant quarantine.

Introduction

Issues of biological and food security of the Russian Federation are associated with the invasive processes of pests. Plant quarantine plays an essential role in the phase of pest prevention, which is ensured by accurate and operational diagnostics (or detection and identification) of pests [1].

An essential aspect of timely detection of alien pests is predicting their potential spread. Reliable prediction of zones of the possible species acclimatization combined with data on possible pathways of distribution ensures early detection of the species in case of its introduction. In modern practice, methods of algorithmic calculation of the ecological niche and the possible area of the species using specialized software are usually used for this purpose [2].

In the present work, in the context of phytosanitary diagnostics, the morphology and ecology of three species of hemipterans (Insecta: Hemiptera): *Chionaspis pinifoliae* (Fitch, 1856), *Lycorma delicatula* (White, 1845), *Megacopta cribraria* (Fabricius, 1798) that pose a phytosanitary risk for the territory of the Russian Federation are considered.

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Materials and Methods

Materials and results of the authors' field research (South Korea, Mexico) were included. The collections of the Zoological Institute of the Russian Academy of Sciences (St. Petersburg, Russia) and the Zoological Museum of Moscow State University (Zoomuseum of Moscow State University, Moscow, Russia), as well as the collections of the All-Russian Plant Quarantine Center (Moscow Region, Russia), were studied. Pest species indicate the volume of the studied material.

The distribution of pests and their regulatory status is given in the EPPO and CABI databases (unless otherwise specified) [3, 4].

Making micro preparations. For Diaspididae, Canada balsam-based staining with fuchsin was performed.

Microscopy and photography. Zeiss Stemi 2000-C and Stereo Discovery.V12 stereo microscopes were used to study insects. A Zeiss Axio Imager A1 microscope equipped with phase-contrast was used to study the micro preparations. Photography and morphometry were performed using Zen 2.3 and Adobe Photoshop CC software.

Area modeling. Building a model of the possible area of *M. cribraria* within Russia, information on the geographic coordinates of its detection sites, and raster cartographic materials [5] containing data on environmental bioclimatic parameters were used. Free software open Modeller version 1.5.0 and Maxent version 3.4.1 were used for algorithmic construction of models.

Results and Discussion

Pine needle scale. *Chionaspis pinifoliae* (Fitch, 1856), Sternorrhyncha: Diaspididae, is a North American pest of conifers, mainly of the genus *Pinus* [6]. Pine needle scale is included on the European-Mediterranean Plant Quarantine Organization's Signal List, EPPO (EPPO is currently conducting a pest risk analysis), and on Chile's list of quarantine facilities. It may pose a phytosanitary risk to the territory of Russia.

There is specific complexity in differentiating species of the *Chionaspis pinifoliae-heterophyllae* complex, which includes at least six species inhabiting the same host plants [7]. One of the key diagnostic signs is the structure of the pygidium median lobes, particularly the ratio of width to length and the distance between them [8]. The present study proposes an approach to quantify the state of this character in *Ch. pinifoliae* (1 specimen from Mexico, Zoological Institute of the Russian Academy of Sciences (ZIN RAS)) and *Ch. heterophyllae* (6 specimens from Mexico, ZIN RAS, VNIIKR). The following parameters were assessed (Fig. 1): depth of notch between lobes (*H*) is the length of the segment drawn at an angle of 90° from the line connecting the tops of lobes; distance between lobes (*L*) at the level h is ⅓ of distance *H* from the line connecting the tops of lobes; width of lobes (*S*), left and right, at level *h*. In addition, we calculated indexes, which are ratios of the above parameters: *L*/*H*, *L/h*, and *L/S* (Table 1).

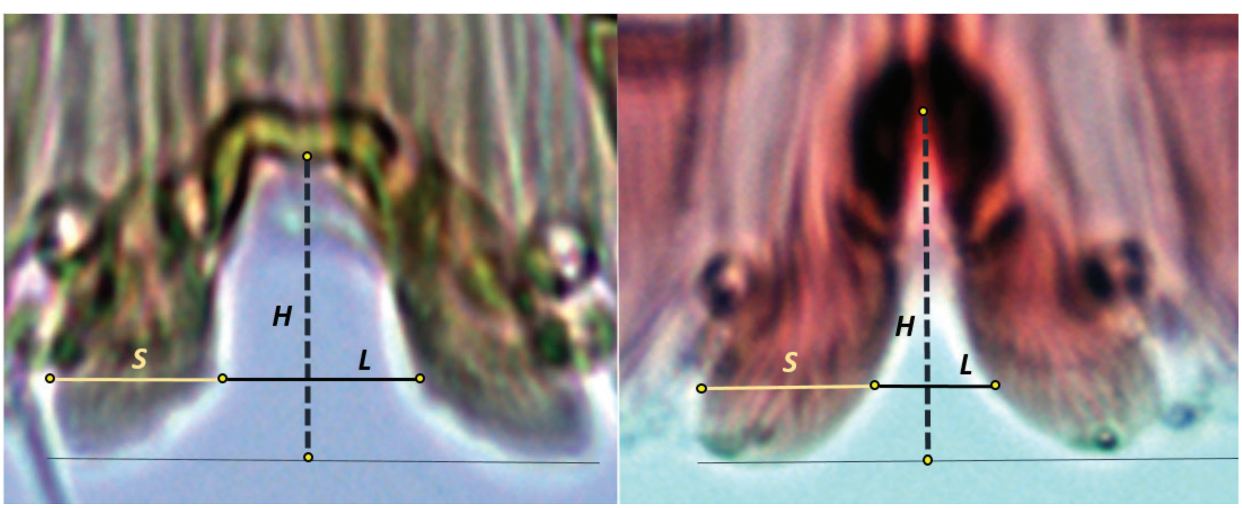

FIGURE 1. Median lobes of *Chionaspis pinifoliae* (left) and *Ch. heterophyllae* (right) and studied morphometric parameters. Explanations are given in the text (photo by A.V. Shipulin)

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TABLE 1. Morphometric parameters (μm) and indexes of the pygidium median lobes of *Chionaspis pinifoliae* and *Ch. heterophyllae.* The mean±standart error of the mean is given (minimum and maximum values of the characteristics in parentheses)

Study species	Parameters					Indexes			
	H	*h*	*L*	*S* left	*S* right	*L/H*	*L/h*	*L/S* left	*L/S* right
Chionaspis pinifoliae n=1	19.2	6.4	4.0	9.1	9.3	0.2	0.6	0.4	0.4
Chionaspis heterophyllae n=6	15.2±0.8 (12.1-17.7)	5.1±0.3 (4.0-5.9)	9.7±0.7 (7.5-12.1)	7.4±0.7 (5.7-10.5)	7.7±0.7 (6.4-11.0)	0.7±0.1 (0.5-0.8)	2.0±0.2 (1.4-2.5)	1.4±0.2 (0.8-1.8)	1.3±0.2 (0.8-1.7)

The results showed that *Ch. pinifoliae* and *Ch. heterophyllae* tended to differentiate by lobe spacing (L) and L/h and L/S indexes (Table 1). The calculation of the L/S index is the same as the Miller, Davidson (2005) approach for diagnosing the species in question. Still, in our case, the measurement procedure is formalized, which has significance for the practice of phytosanitary laboratories. A reference specimen of *Ch. pinifoliae*, deposited in the collection of the ZIN RAS, has been isolated.

Spotted lanternfly. *Lycorma delicatula* (White, 1845), Auchenorrhyncha: Fulgoridae, is a polyphagous, noted on 70 plant species, including economically significant horticultural, ornamental, and forest crops. Over the past two decades, the species has shown itself to be an invader, entering from China into Korea (2004) and the United States (2014), where it causes economic damage to grape production.

The study of the collection of insects of the Zoological Museum of Moscow State University (Zoomuseum of Moscow State University) showed that 83 specimens of Fulgoridae belong to 6 subfamilies: Aphaeninae, Poiocerinae, Dichopterinae, Fulgorinae, Phenacinae, and Zanninae. Species of tribes Limosini and Aphaenini represent subfamily Aphaeninae. The latter tribe includes *L. delicatula*, specimens of which are deposited in the Zoomuseum of Moscow State University (isolated as reference specimens); in addition, this species was studied by the authors in its secondary area in South Korea (Fig. 2).

In the genus Lycorma, in addition to *L. delicatula,* three other species are well distinguished by the pattern and coloring of the wings [9]. However, identification at the subspecies taxon level is complex and requires appropriate research to develop a diagnostic protocol.

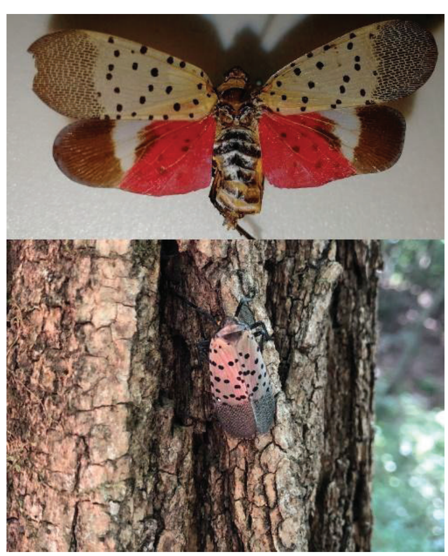

FIGURE 2. *Lycorma delicatula: top* – unfurled specimen from the Zoomuseum of Moscow State University (photo by J. A. Lovtsova), *below* – species in natural conditions, South Korea (photo by I.O. Kamayev)

Bean plataspid. *Megacopta cribraria* (Fabricius, 1798), Heteroptera: Plataspidae. This bug is a serious pest of soybeans in East and South Asia and has also entered North America and is already widely distributed in the United States [10]. In this paper, an attempt was made to model the potential distribution of *Megacopta cribraria* in Russia.

Modeling results showed a high probability of distribution under existing climatic conditions of the species only in the south of Krasnodar Krai and the Republic of Adygea, where about 10% of soybean homegrown is concentrated [11], and a low one within the North Caucasus from the Black Sea coast to the Chechen Republic. Area modeling under existing climate change models (ssp126, ssp585) showed the probability of insignificant decrease of the potential area of species distribution in the Northern Caucasus by the end of the XXI century and an increase of the acclimatization probability in the south of the Far East (but remaining low).

The hypothesis was confirmed [12] that the main climatic factors determining the potential distribution of *M. cribraria* include minimum temperatures and total precipitation. In addition, the uniformity of temperatures throughout the year and the dry periods' absence has been shown to have a high impact.

Prediction of the *M. cribraria* potential area and methods of identification of this species probably need refinement due to the presence of a taxon with disputable status – *Megacopta punctatissima* (Montandon, 1896). Although most modern taxonomists consider this taxon a junior synonym of *M. cribraria* [13], some authors believe that this species is independent. Distinguishing these forms by morphological methods is problematic; molecular genetic methods [14] also showed the intermediate forms with disputable status. *M. punctatissima* may be only a trophic form of *M. cribraria* [15]. Because of this, both forms were treated as one species in the simulation experiments. In the case of new data showing the validity of *M. punctatissima* species, the results of modeling the ecological niche and area of *M. cribraria* will require revision.

Conclusion

The results of studies on the phytosanitary biology of three hazardous species of hemipterans led to the following conclusions:

1. Approaches to morphometric studies of pygidium diagnostic signs of the complex *Chionaspis pinifoliae-heterophyllae* to differentiate *Chionaspis pinifoliae* from other species and to formalize the measurement procedure, which is essential for the practice of phytosanitary laboratories, were proposed;
2. *Lycorma delicatula* is well-differentiated from other species of the genus, with identification at the level of supraspecific taxa being complicated and requiring appropriate research;
3. The simulation results of the *Megacopta cribraria* area for Russia showed a high probability of its distribution within the south of Krasnodar Krai and the Republic of Adygea, which should be considered when identifying this pest in case of its possible infestation.

Reference specimens of the following species were isolated: *Chionaspis pinifoliae* deposited in the Zoological Institute of the Russian Academy of Sciences (St. Petersburg, Russia) and *Lycorma delicatula* from the collection of the Zoomuseum of Moscow State University (Moscow, Russia). The latter is of practical importance for developing and applying diagnostic protocols in plant quarantine. In particular, this practice exists in developing and revising diagnostic protocols for insects and mites by the EPPO Panel Diagnostics in Entomology.

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Scientific Monitoring of Leguminous Crops Cultivated Areas in the Russian Federation

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Abstract. The soybean bacterial wilt causative agent *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* (Hedges) Collins & Jones, the halo blight of bean causative agent *Pseudomonas savastanoi* pv. *phaseolicola* (Burkholder) Gardan et al., the common blight of bean causative agent *Xanthomonas axonopodis* pv. *phaseoli* (Smith 1897) Vauterin et al., and bacterial blight of pea causative agent *Pseudomonas syringae* pv. *pisi* (Sackett) Young et al. are frequently detected diseases of leguminous crops, they cause severe production losses, show a certain level of field adaptation in leguminous crops, persist on the surface and inside the seeds without losing their viability for a long time. These features led to the penetration probability of leguminous crops bacteriosis with imported products under control. The phytosanitary risk of bacteriosis of soybeans, beans, and peas, along with the globalization of trade, requires the development of precise diagnostic tests, emphasize the use of quarantine inspections to determine the bacteriosis area and to prevent disease extension in leguminous crops production. This paper presents a part of research being conducted to study the spread of bacteria infecting leguminous crops and to determine the zones free of the bacteriosis at the Test Laboratory Center of the All-Russian Plant Quarantine Centre (FGBU VNIIKR). Some available molecular diagnostic methods (real-time PCR and conventional PCR) have been tested and shown to be sufficiently effective for pathogen detection and identification. At the same time, the surveys of the crop fields indicated the applicability for additional research using alternative detection and identification methods to confirm the presence of causative agents *C. f.* pv. *flaccumfaciens*, *X. a.* pv. *phaseoli*, and *P. s.* pv. *pisi* in the analytical samples to determine the cultivated areas with approved bacterial phytopathogens of leguminous crops in the Russian Federation.

Keywords: *Curtobacterium flaccumfaciens* pv. *flaccumfaciens*, *Pseudomonas savastanoi* pv. *phaseolicola*, *Xanthomonas axonopodis* pv. *phaseoli*, *Pseudomonas savastanoi* pv. *pisi*, conventional PCR, RT-PCR.

INTRODUCTION

Production, trade, and consumption of grain legume crops have increased significantly over the past fifteen years. There is an urgent need to close the large gap between potential and actual yields. This, in turn, requires a significant agricultural research effort to keep grain products free of bacterial infections [1, 2]. World practice emphasizes the use of quarantine inspections to determine the bacteriosis area and to prevent disease spreading in legume grain production [3].

The study objects are the soybean bacterial wilt causative agent *Curtobacterium flaccumfaciens* pv. *flaccumfaciens*, the halo blight of bean causative agent *Pseudomonas savastanoi* pv. *phaseolicola*, the common blight of bean causative agent *Xanthomonas axonopodis* pv. *phaseoli,* and the bacterial blight of pea causative agent *Pseudomonas syringae* pv. *pisi*. The research results in modern world practice show that the studied bacteriosis isolates in grain legume crops show a certain level of field adaptation [4-13]. This is primarily caused by to the migration and spread way of bacteriosis, as well as the wide range of host plants: *Phaseolus vulgaris*, *Phaseolus coccineus*, *Phaseolus lunatus*, *Glycine max*, *Pisum sativum*, *Vigna unguiculata*, *Vigna angularis*, *Vigna umbellate*,

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Vigna aconitifolia, *Vigna radiata*, *Vigna mungo*, *Lupinus polyphyllus*, *Lablab purpureus*, *Vicia sativa*, *Vicia benghalensis*, *Lathyrus latifolius*, *Lathyrus odoratus*, *Cassia hirsuta*. It is necessary to consider the possibility of penetration of grain legume crops bacteriosis with imported products under control. Phytopathogens persist on the surface and inside the seeds without losing their viability under extremely adverse environmental conditions. The causative agents of bacterial phytopathogens can persist in plant residues and weeds and the soil during the unfavorable for bacteria winter period for at least two years in legume-wheat rotation.

Rapid and accurate detection of bacteria in seed batches would reduce the risks of sowing infected grain in an environment favorable to the reproduction of bacterial causative agents [10, 13, 14]. The phytosanitary risk of bacteriosis of soybeans, beans, and peas, combined with the globalization of trade, requires the introduction of highly specific diagnostic tests that will be regularly and easily used at official import ports and in the fields [13-16].

During 2019-2021, the Federal Service for Veterinary and Phytosanitary Surveillance of the Russian Federation (Rosselkhoznadzor) has introduced a requirement to develop methodological recommendations (as a temporary quarantine phytosanitary measure) for detecting and identifying the soybean bacterial wilt causative agent *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* (Hedges) Collins & Jones (No. 49-2019 MR VNIIKR), the halo blight of bean causative agent *Pseudomonas savastanoi* pv. *phaseolicola* (Burkholder) Gardan et al. (No. 38-2019 MR VNIIKR), the bacterial blight of pea causative agent *Pseudomonas syringae* pv. *pisi* (Sackett) Young et al. (currently, the reference document is under development). Given the bacteriosis penetration probability into the territory of the country in the period preceding the development of methodological recommendations, it is necessary to conduct scientific monitoring of leguminous crops cultivated areas in the Russian Federation for the purpose:

- studying the spread of bacteria infecting grain legume crops;
- determining of areas free from the bacteriosis under study.

Materials and Methods

The paper uses plant material from the survey of grain legume crops in the Russian Federation in 2019-2021.

Visual inspection was carried out, and in the presence of symptoms (characteristic of bacteriosis of grain legume crops), the plant samples were taken during the period from May to September. Leaves and stems of plants were selected as a material.

The following is used in a study as positive control sample (PCS):

- a standard strain of the bacterium NCPPB 1446 *C. f. flaccumfaciens* from The National Collection of Plant Pathogenic Bacteria (NCPPB), York, UK;
- referent strains *P. s. phaseolicola*: CFBP1390, CFBP1429, CFBP3652 from the Collection Française de Bactéries Phytopathogènes (CFBP), Paris, France;
- a referent strain *X. a. phaseoli* CFBP2534;
- a referent strain of the bacterium CFBP 2105 *P. s.* pv. *pisi*.

Phytopathogens from the bacteriological collection of phytopathogenic strains from the All-Russian Plant Quarantine Centre were used as non-target organisms in the study.

DNA extraction was performed using commercial kits according to the manufacturer's instructions: "Proba-GS" OOO "AgroDiagnostics", based on the sorption method of DNA isolation, and "M-Sorb-Tub-Automat" (ZAO "Sintol"), based on the use of magnetic particles, the extraction process is robotized (nucleic acid extraction station "Freedom EVO" Switzerland). Conventional PCR and RT-PCR were performed with the DNA isolated from the samples.

Conventional PCR according to Tegli et al. [5, 15, 16]. The method detects C. f. pv. flaccumfaciens DNA using the forward primer CffFOR2 and the reverse primer CffREV4. The 25 µl of reagent mixture for a single reaction contain: 5 µl – 5× PCR reaction mixture, 2 µl – CffFOR2, 2 µl – CffREV4, 2.5 µl – DNA, 13.5 µl – PCR water. The temperature and time parameters of conventional PCR for primers CffFOR2 and CffREV4 included: primary denaturation at 94°C – 3 min; then 30 cycles (94°C – 1 min, 62°C – 45 sec, 72°C – 30 sec) 72°C – 5 min.

RT-PCR according to Min Seok Cho et al. [7]. The method detects *P. s.* pv. *phaseolicola* DNA using the forward primer SSRPF, reverse primer, and probe SSRPP. The 20 µl of reagent mixture for a single reaction contain: 4 µl – 5× PCR reaction mixture, 1 µl – SSRPF, 1 µl – SSRPR, 1 µl – SSRPP, 2 µl – DNA, 11 µl – PCR water. RT-PCR temperature and time parameters for primers and a probe SSRPF, SSRPR, and SSRPP include primary denaturation at 95°C – 3 min; then 45 cycles (95°C – 10 sec, 61°C – 20 sec).

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RT-PCR, according to Boldwin et al. [10, 11, 14]. The method detects *X. a.* pv. *phaseoli* DNA using the forward primer AuF, reverse primer AuR, and probe AuP. The 25 µl of reagent mixture for a single reaction contain: 5 µl – 5× PCR reaction mixture, 1 µl – AuF, 1 µl – AuR, 1 µl – AuP, 5 µl – DNA, 13 µl – PCR water. RT-PCR temperature and time parameters for primers and a probe AuF, AuR, and AuP include primary denaturation at 95°C – 5 min; then 40 cycles (95°C – 1 min 60°C – 40 sec).

Conventional PCR, according to Qing et al. [4]. The method detects *P. s.* pv. *pisi* DNA using the forward primer AN7F and reverse primer AN7R. The 25 µl of reagent mixture for a single reaction contain: 5 µl – 5× PCR reaction mixture, 0,6 µl – AN7F, 0,6 µl – AN7R, 2 µl – DNA, 16.8 µl – PCR water. The temperature and time parameters of conventional PCR for primers AN7F and AN7R include: primary denaturation at 94°C – 5 min; then 35 cycles (94°C – 30 min, 55°C – 30 sec, 55°C – 30 sec, 72°C – 30 sec) 72°C – 5 min.

For RT-PCR, a thermal cycler for PCR DT-Prime (DNA-Technology, Russia) was used; for classical PCR, a C 1000 Tourch thermocycler (Bio-Rad, USA) was used. Amplification results were recorded after electrophoresis on 1.5% agarose gel using GeneRuler™ 100+ bp molecular weight marker. (Thermo Fisher Scientific, USA).

Results

From 2019 to 2021, molecular diagnostic methods were used to analyze analytical samples of grain legume crops material (656 samples) obtained during the survey of cultivated areas in the Altai, Trans-Baikal, Primorye, and Khabarovsk Territories; Amur, Belgorod, Voronezh, Kursk, Orel, Rostov, Saratov, and Tomsk regions; the Jewish Autonomous Region (Fig. 1).

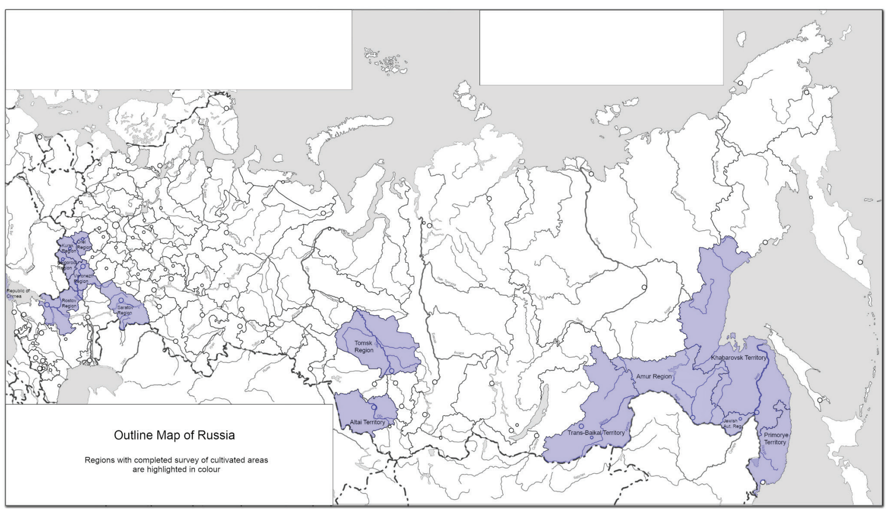

FIGURE 1. A contour map of the 2019 and 2021 Russian cultivation fields survey for the presence of leguminous crops phytopathogens. Source: Compiled by the authors

During the analyses on leguminous crops detection, according to Tegli et al., a positive result was obtained using conventional PCR for one sample from Primorye Territory in 2019. According to Min Seok Cho et al., negative results were acquired using RT-PCR in all 656 samples. According to Boldwin et al., a positive result was obtained by RT-PCR for one sample from the Amur region in 2019. According to Qing et al., positive results were obtained conventional PCR by for one sample from the Kursk region in 2020 and one from the Belgorod region in 2020.

Thus, we determined the presence of genetic material of *C. f.* pv. *flaccumfaciens*, *X. a.* pv. *phaseoli*, and *P. s.* pv. *pisi* in 4 analytical samples during the scientific monitoring of grain legume crops cultivated areas in Russia in 2019-2021.

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Discussion

C. f. pv. *flaccumfaciens*, *P. s.* pv. *phaseolicola*, *X. a.* pv. *phaseoli*, and *P. s.* pv. *pisi* detection and identification is approved in all samples by means of primers and probes for RT-PCR and primers for conventional PCR using the investigated method to prepare analytical samples.

As it can be seen from the results, when surveying the leguminous crops cultivation areas in Russia, we can enumerate a list of areas free of bacteriosis causative agents *C. f.* pv. *flaccumfaciens*, *P. s.* pv. *phaseolicola*, *X. a.* pv. *phaseoli* и *P. s.* pv. *pisi*. Phytopathogens were not detected in the Altai, Trans-Baikal, and Khabarovsk Territories; the Voronezh, Orel, Rostov, Saratov, and Tomsk Regions; the Jewish Autonomous Region.

Conclusion

It is necessary to carry out additional investigations with the help of detection and identification methods to confirm the presence of causative agents *C. f.* pv. *flaccumfaciens*, *X. a.* pv. *phaseoli*, and *P. s.* pv. *pisi* in the 4 analytical samples to enumerate the cultivation areas with the presence of bacterial phytopathogens of leguminous crops in the Russian Federation.

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Test of the Molecular Genetic Methods for Diagnosing Tomato Bacterial Cancer

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Abstract. Tomato bacterial cancer is widespread in the world and causes significant damage in tomato cultivation. Infected seeds are the main way of spreading bacteriosis over long distances. The European and Mediterranean Plant Protection Organization has included the causative agent of tomato bacterial cancer in the list of quarantine objects in List A2. Diagnostics of the phytopathogen at all stages of tomato production will make it possible to take timely measures to protect plants, which will significantly reduce crop losses and increase the economic efficiency of domestic fruit production. The existing diagnostic schemes for the disease imply large labor costs and a long research period, which is unacceptable in routine laboratory work. The article compares two diagnostic methods based on real-time polymerase chain reaction according to: Bach et al. (2003) and Oosterhof & Berendsen (2011). The research aims to assess the inhibition of PCR by direct isolation of bacterial DNA from plant extracts. PCR according to Bach et al. (2003) and the internal positive control synthesized by the domestic company LLC Syntol were selected for further research. The studies were carried out on the basis of the All-Russian Plant Quarantine Center (FSBI "VNIIKR").

INTRODUCTION

In the context of active government support for economic entities in recent years, there has been an increase in the production of greenhouse tomatoes, which is primarily associated with the policy of import substitution. So, in the Russian Federation in 2016-2019, the gross harvest of tomatoes grown in the open field increased by 20.2% (from 1.73 to 2.08 million tons), in greenhouses – by 56.7% (from 0.60 to 0.94 million tons). In the context of the coronavirus pandemic in 2020, the gross harvest of tomatoes decreased by 3.3% compared to 2019 and amounted to 2.92 million tons [1].

The result of improving technologies for growing tomato in greenhouse agrobiocenosis is the creation of favorable conditions for the development of not only plants, but also phytopathogenic microorganisms. Under these conditions, bacterial diseases, in particular, bacterial tomato cancer, exhibit increased harmfulness [2]. Tomato yield losses can be up to 30-50% [3].

Phytopathogenic bacterium *Clavibacter michiganensis* subsp. *michiganensis* (Cmm) is one of the most dangerous tomato pathogens worldwide [4-6].

Despite its widespread distribution throughout the world, many regional organizations have included Cmm on the A2 List of Quarantine Pests [7]. Most likely this is due to the purpose of controlling the causative agent of tomato bacterial cancer in greenhouses, which will subsequently contribute to the improvement of tomato culture in the regions.

Bacteriosis is transmitted by seeds, both as an internal and superficial infection. Latently infected seedlings are also the cause of the spread of the pathogen [4, 8, 9].

The main and effective measure for the control of bacterial tomato cancer is the use of seeds and seedlings free from the causative agent of the disease [5, 10].

Thus, timely and high-quality diagnostics of the causative agent of tomato cancer in seeds and seedlings can help reduce crop losses and increase the economic efficiency of the production of domestic fruits.

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Today, in the international practice of Cmm diagnostics, the following documents are used: the EPPO regional standard RM 7/42 (3) [4], as well as test reports: "Method for the Detection of *Clavibacter michiganensis* subsp. *michiganensis* on Tomato seed" [11] and "Detection of Clavibacter michiganensis subsp. michiganensis on Tomato seed" [5].

In PM 7/42 (3), two diagnostic schemes are proposed, one for the vegetative parts of plants, the other for the seed material. It is worth noting that the first regimen was developed for symptomatic samples only. Therefore, this approach cannot be used to diagnose a latent form of infection. The rest of the protocols propose schemes for detecting the pathogen from seed only. Moreover, all laboratory schemes provide for mandatory preliminary isolation of bacteria on semi-selective nutrient media, followed by screening and identification of suspicious colonies using molecular methods. In the test protocol [11], when examining samples, it is recommended to conduct additional studies to assess the possible manifestation of antagonism.

In 2018, studies showed that some Cmm bacteria, in response to unfavorable factors, stop growing on agar nutrient medium, but retain their viability [12].

Thus, the main disadvantages of the proposed schemes for detecting and identifying Cmm are: the duration of research (from the moment of obtaining plant extracts to the appearance of typical colonies on nutrient media, it takes about 5-10 days); the need to take into account the possibility of antagonism [11] and the uncultivated state of Cmm.

Currently, PCR analysis has replaced the classical approach as a routine method for diagnosing bacterial plant diseases.

The research process can be shortened by direct isolation of DNA from extracts [13]. However, with this approach, there is a risk of PCR inhibition.

Thus, the purpose of these studies was to assess the inhibition of PCR in an experiment with artificially infected extracts of vegetative parts and seeds of tomato.

Materials and Methods

Preparation of analytical samples from vegetative parts of tomato. We used the following varieties of tomatoes: "Beysuzhek" F1 and "Cherry red". The leaves and stems were finely chopped into pieces (approximately 0.5 cm). 8 weighed portions of two options were prepared: 1 g and 2 g in size. Weighed portions were placed in sterile plastic containers with a volume of 120 ml and filled with 30 ml of sterile PBS. Then the containers were transferred to an orbital shaker for 1 hour (mode – 200 rpm) at room temperature. Then, filtration was carried out through a paper filter into Oak Ridge centrifuge tubes (Nalgene, Thermo Fisher Scientific, USA) and centrifuged on an Allegra X-30 device (Beckman Coulter, USA) for 10 min at a speed of 10000 g at 10 °C. The supernatant was carefully discarded. The precipitate was dissolved in 1 mL of sterile PBS buffer by shaking thoroughly on a vortex. Thus, 8 ml were prepared.

Artificial infection of tomato vegetative parts. Artificial infection of prepared extracts was carried out with a bacterial suspension of a pure culture of the Cmm (0240) strain from the collection of the laboratory of bacteriology and analysis of the GMO of FSBI VNIIKR. Eight consecutive tenfold dilutions were prepared in two versions of the extract and in sterile PBS.

Serial dilutions in PBS were used to quantify microorganisms. On a solid nutrient medium YDC, 5, 6, and 7 dilutions of the culture in sterile PBS were inoculated in duplicate, 100 µl each. On the 3rd day of incubation, the colonies on the plates were counted and the number of CFU/ml was determined in zero and subsequent dilutions.

Artificial seed contamination. A 10 g sample of seeds was placed in a bag for homogenization, 20 ml of Cmm bacteria suspension in sterile PBS buffer ($\approx 10^6$ CFU/ml) was poured and placed in an incubator shaker for 4 hours at 10 °C and 90 rpm. Then the excess suspension was poured through a paper filter and the seeds were dried at room temperature for 20 hours. Four more weighed portions of seeds were prepared, measuring 7.2 g, 7.6 g, 7.92 g, and 8 g. The first three were mixed with infected seeds, so that each weighed portion was equal to 8 g. As a result, 5 variants of seeds with different levels of infection were obtained: 100%, 10%, 5%, 1% and uninfected seeds.

Preparation of analytical samples from seeds. Samples of five variants of the experiment were prepared by the homogenization method [4].

DNA purification. Infected extracts in an amount of 200 µl were used for DNA isolation using a commercial kit "Proba GS" (LLC "AgroDiagnostika", Russia). The method is based on the principle of tissue lysis in a guanidine thiocyanate (GuSCN) solution with the adsorption of total DNA on the surface of the sorbent (silicon dioxide) and subsequent three-fold washings with appropriate solutions.

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PCR analysis. To detect and identify Cmm DNA, we used two pairs of primers and probes developed by Bach et al. (2003) [5, 14] and Oosterhof & Berendsen (2011) [4, 11, 15]. To assess the inhibition of the PCR reaction, we used an internal positive control (IPC) synthesized by the Russian company LLC Syntol.

Bach et al. developed PCR for quantitative assessment and differentiation of phytopathogenic species of the genus *Clavibacter*. Primers FP_Cm/RP_Cm were selected for the region of the intergenic sequence of the rRNA operon (ITS), common to all species listed below: *C. sepedonicus, C. michiganensis* subsp. *michiganensis, C. tessellarius, C. nebraskensis, C. insidiosus*. The specificity of the method is achieved by means of probes developed for each pathogenic species of the genus *Clavibacter*. The authors noted the high specificity of the test system, but there is no information on the analytical sensitivity. The composition of the reaction mixture for one test sample is as follows: deionized water – 12.0 µl, 5X PCR mix – 5 µl, FP_Cm – 1 µl, RP_Cm – 1 µl, Cmm Probe 6FAM – 1 µl, total DNA – 5 µl, total volume – 25 µl. Amplification program: 1) 95 °C – 10 min; 2) 45 cycles: 95 °C – 20 sec, 66 °C – 1 min.

PCR according to Oosterhof & Berendsen (2011) was developed based on the sequence of a gene region encoding a two-component systemic sensory kinase (PTSSK). The authors note the sensitivity of the method at the level of 2 x 103 CFU/ml and the absence of both false-negative and false-positive results. The composition of the reaction mixture for one test sample is as follows: deionized water – 16.5 µl, 5X PCR mix – 5 µl, RZ_ptssk 10 – 0.5 µl, RZ_ptssk 11 – 0.5 µl, Probe RZ_ptssk 12 – 0.5 µl, total DNA – 2 µl, total volume – 25 µl. Amplification program: 1) 95 °C – 10 min; 2) 40 cycles: 95 °C – 15 sec, 60 °C – 30 sec.

For setting both PCR, a ready-made mix for PCR 5x MasCFEMix-2025 (Dialat Ltd., Russia) and a CFX96 C1000 Touch device (Bio-Rad Laboratories, Inc., USA) were used.

Results

RT-PCR results for detecting Cmm from vegetative parts of tomato according to Bach et al. (2003) and Oosterhof & Berendsen (2011) are shown in Tables 1 and 2.

TABLE 1. RT-PCR results according to Bach et al. (2003)

Dilutions (number of cells, CFU/ml)	Replications	Weighing 1 g		Weighing 2 g	
		Cq, FAM	Cq, HEX	Cq, FAM	Cq, HEX
1 (9.5×10^7)	1	23.30	34.31	23.55	35.12
	2	23.43	37.72	23.59	38.85
	3	23.16	35.22	23.50	36.16
2 (9.5×10^6)	1	26.49	35.93	26.58	37.55
	2	26.60	44.10	26.60	36.45
	3	26.37	38.87	26.55	36.56
3 (9.5×10^5)	1	30.53	34.39	30.32	35.53
	2	30.59	35.93	30.54	39.60
	3	30.46	34.35	30.10	37.94
4 (9.5×10^4)	1	33.36	40.15	33.39	36.27
	2	33.30	44.43	33.33	40.22
	3	33.41	40.12	33.44	38.43
5 (9.5×10^3)	1	36.47	36.29	37.12	34.08
	2	36.30	36.97	36.35	36.89
	3	36.64	36.33	37.89	34.76
6 (9.5×10^2)	1	-	34.89	-	39.00
	2	-	36.39	-	43.87
	3	-	36.43	-	40.52
7 (9.5×10^1)	1	-	36.39	-	35.74
	2	-	38.72	-	40.16
	3	-	38.23	-	42.83
8 (9.5×10^0)	1	-	35.96	-	35.72
	2	-	36.82	-	35.96
	3	-	35.74	-	36.71

Source: Compiled by the authors

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TABLE 2. RT-PCR results according to Oosterhof & Berendsen (2011)

Dilutions (number of cells, CFU/ml)	Replications	Weighing 1 g		Weighing 2 g	
		Cq, FAM	Cq, HEX	Cq, FAM	Cq, HEX
1 (9.5 x 10^7)	1	28.72	34.68	28.76	36.28
	2	28.69	34.33	28.14	39.12
	3	28.75	35.02	28.62	33.43
2 (9.5 x 10^6)	1	32.78	35.42	31.54	37.68
	2	32.62	36.37	31.71	-
	3	32.94	24.47	31.36	35.24
3 (9.5 x 10^5)	1	35.29	33.20	34.76	-
	2	35.40	-	36.52	36.05
	3	35.18	36.37	38.99	38.53
4 (9.5 x 10^4)	1	38.56	37.22	-	34.61
	2	37.98	38.02	-	34.15
	3	39.17	36.20	-	35.06
5 (9.5 x 10^3)	1	-	33.48	-	36.27
	2	-	-	-	36.06
	3	-	34.22	-	36.47
6 (9.5 x 10^2)	1	-	-	-	-
	2	-	-	-	-
	3	-	-	-	-
7 (9.5 x 10^1)	1	-	38.23	-	36.02
	2	-	37.04	-	37.30
	3	-	-	-	34.74
8 (9.5 x 10^0)	1	-	33.18	-	36.30
	2	-	32.30	-	38.97
	3	-	34.05	-	33.63

Source: Compiled by the authors

Stable results of detection of target DNA (FAM) in the first version (1 g sample) were noted from 1 to 4 dilutions, in the second version (2 g sample) – from 1 to 3 dilutions. Thus, the analytical sensitivity for the first option was 9.5 x104, for the second – 9.5 x105. It was also noted that the internal control (HEX) did not work in 11 reactions, indicating the presence of PCR inhibitors.

The results of artificial infection of tomato seeds are presented in Table 3.

TABLE 3. Results of artificial infection of tomato seeds

Infestation, %	Replications	PCR according to Bach et al. (2003)		PCR according to Oosterhof & Berendsen (2011)	
		Cq, FAM	Cq, HEX	Cq, FAM	Cq, HEX
100	1	18.98	34.34	25.11	28.62
	2	19.02	34.12	25.04	28.55
	3	18.72	34.62	24.96	28.49
10	1	22.53	33.55	28.98	28.32
	2	22.47	33.72	28.86	28.54
	3	22.34	33.47	28.47	28.63
5	1	22.93	33.79	29.91	28.42
	2	23.04	33.56	30.17	28.67
	3	22.98	33.83	29.89	28.38
1	1	26.10	34.65	-	28.56
	2	26.76	33.96	-	28.43
	3	26.53	33.78	-	28.54
0	1	-	33.57	-	28.34
	2	-	33.68	-	28.55
	3	-	33.59	-	28.46

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Source: Compiled by the authors

The table shows the detection of Cmm when testing RT-PCR in accordance with Bach et al. (2003) noted at all levels of seed infestation. When testing RT-PCR in accordance with Oosterhof & Berendsen (2011), Cmm was not detected at an infection level of 1%. Internal control worked in all reactions, indicating no inhibition.

Discussion

When testing RT-PCR for detecting Cmm from the extract of vegetative parts of tomato, PCR according to Bach et al. (2003) showed stable results of detection of target DNA (FAM) in both variants from 1 to 5 dilutions, IPC worked in all reactions; PCR in accordance with Oosterhof & Berendsen (2011) – in the first version (1 g sample), 1 to 4 dilutions were noted, in the second version (2 g sample) – from 1 to 3 dilutions, IPC did not work in 11 reactions, which indicates for the presence of PCR inhibitors.

When testing RT-PCR for detecting Cmm from tomato seed extract, RT-PCR according to Bach et al. (2003) passed at all levels of seed infestation. When testing RT-PCR in accordance with Oosterhof & Berendsen (2011), Cmm was not detected at an infection level of 1%. Internal control worked in all reactions, indicating no inhibition.

Prior to the detection of Cmm from plant extracts, RT-PCR can be recommended according to Bach et al. (2003).

Conclusion

To establish the degree of influence of the plant matrix on the final results of studies of samples of plants and seeds of tomato, we carried out experiments on artificial infection with the causative agent of tomato bacterial cancer of extracts from vegetative parts and seeds of tomato. In the course of testing the method for preparing samples with further DNA isolation and PCR analysis of artificially infected samples, it was found that there was no PCR inhibition.

The studies carried out have shown the possibility of using RT-PCR for the direct detection of Cmm from plant extracts; however, first it is necessary to determine the criteria for the effectiveness of the tests under study.

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Improvement of Diagnostic Methods for the Most Dangerous Viruses of Grain Crops

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Abstract. Grain crops are a key group of agricultural plants. Due to the increasing demand for grain products in the world and the dynamics of grain industry development, Russia has all the necessary resources for the growth of grain production. Grain farming accounts for one-fifth of all agricultural production costs and about 50% of the profits of agricultural producers. The main grain crops on the world market are wheat Triticum L., barley Hordeum L., oats Avena L., corn Zea mays L. In terms of wheat production, Russia ranks 5th place with a share of more than 5% in world production. Russia is a net exporter of wheat: exported volume exceeds imported volume by an order of magnitude. In terms of wheat exports, Russia ranks 1st place in the world. In 2018, exports reached a record high of 44 MMT worth $8bn. Corn (Zea mays L.) is one of the most common grain crops in the world. It ranks third in terms of planted areas in the world. Planted areas for grain corn in Russia in 2018, according to Rosstat, amounted to 2,452.2 ths ha. In 2018, 11,162.6 TMT of corn were harvested. Barley (Hordéum vulgáre) is the most important agricultural crop. Barley grain is widely used for food, technical and feed purposes. Barley is one of the most valuable concentrated animal feeds, as it contains high-grade protein and is rich in starch. In Russia, up to 70% of barley is used for fodder purposes. According to Rosstat, barley cultivation area in 2019 amounted to 8,786.9 ths ha. Oats, like barley, are among the most important grain crops. In its pure form and mixed with annual legumes, it is cultivated for grain, green fodder, hay, silage, and grazing. The increasing phytosanitary requirements of importing countries are tightening the requirements for grain quality control. In this regard, it is necessary to develop diagnostic methods for phytopathogens infecting grain crops and included in the phytosanitary requirements of many grain-importing countries. The reasons for the increase in the spread of viruses and diseases caused by them are the intensification of grain cultivation, a more active exchange of seed material, and, consequently, a clearer registration of viruses based on modern immunological and molecular genetic diagnostics is necessary. According to analysis results the main phytosanitary requirements of the importing countries, the most important viruses of grain crops that create risks of banning the import of a significant amount of products have been selected in All-Russian Centre of Plant Quarantine, and methods for diagnosing the most dangerous viruses of grain crops are being developed [1, 2]. The applicability of molecular diagnostic methods of Maize dwarf mosaic virus and Barley stripe mosaic virus has been evaluated. The best reagents and conditions of their application for the diagnosis of the studied viruses have been selected. The selected methods showed high results with samples of grain and herbage of host plants.

INTRODUCTION

Grain crops play a key role in the agriculture of the Russian Federation. Wheat, corn, and barley are among the most economically significant crops for the Russian Federation. These crops are important both for domestic consumption and for exports, which amount to tens of millions of tons per year.

According to the expert-analytical center of agricultural business AB-centre, corn crops averaged 2.5-3 mln ha in 2017-2019. Over the past 10 years, the area under corn has grown almost 2 times, and over 20 years — from the initial 600 ths ha, the growth was more than 4 times. Corn gross yield, after a slight decrease in 2017 and 2018, began to recover, and in 2019 amounted to 13.9 MMT.

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Wheat planted areas in 2019 amounted to more than 28 mln ha, which was the maximum in 10 years. Wheat gross yields in 2019 amounted to more than 74.3 MMT.

Barley planted areas in 2019 amounted to more than 8.7 mln ha, having increased by 780 ths ha since 2017. Barley gross yield in 2019 amounted to more than 20.4 MMT [3].

Oats planted areas in Russia in 2020 amounted to 2.5 mln ha. Oats gross yield in the current year is projected at the level of the previous year — about 4.4 MMT.

The possibility of exporting Russian grain products is significantly influenced by the phytosanitary requirements of importing countries [4]. For unhindered grain exports, it is necessary to comply with such requirements, which include the absence of viruses, including Maize dwarf mosaic virus (MDMV) and Barley stripe mosaic virus (BSMV).

MDMV is a typical representative of Potyviridae family and Potyvirus genus. MDMV virions have a filamentous shape with a length of about 750 nm and a width of 13 nm. A single-stranded RNA strand makes up approximately 5% of viral particle weight, which corresponds to 3320 kDa. Genomic RNA encodes a single polypeptide with a molecular weight of 30.7 kDa. MDMV is part of the Sugarcane mosaic virus (SCMV) subgroup. The host plants include such crops as corn (Zea mays), sorghum (Sorghum bicolor), and sugar cane (Saccharum officinarum). Common millet (Panicum miliaceum) is one of the cultivated plants that can be infected as well.

Barley stripe mosaic virus (BSMV) is the typical species of Hordeivirus genus of Virgaviridae family. BSMV forms rigid rod-shaped virions with a spiral organization of viral coat protein. Virions have a diameter of about 20 nm. The length of virions varies from 100 to 150 nm depending on the size of the encapsidated RNA. The main host plants of BSMV are barley (Hordeum vulgare) and wheat (Triticum aestivum, Triticum durum). BSMV infection on oats (Avena sativa) is less common.

Both viruses can spread by seeds. It may serve as an obstacle to the export of grain products.

MDMV and BSMV are widespread in many countries of the world. According to Agricultural Atlas (www.agroatlas.ru), these viruses are common in the southern regions of Russia. Data on their distribution in the Russian Federation date back to the 1960s and 1970s, which requires assessment by phytosanitary monitoring of the country's territory.

The studied viruses are included in the phytosanitary requirements of some importing countries, such as China and Sudan. In this regard, it is important to develop a fast and accurate diagnostic system that allows detecting the presence of these viruses in grain during pre-export control. The work objective of this study was to optimize the published molecular methods for the diagnosis of two viruses, pathogens of grain crops.

Materials and Methods

The work used samples of plant seeds collected in 2019-2020 in Rostov region, the Kabardino-Balkarian Republic, Moscow region, and Krasnodar Krai.

RNA isolation was carried out with a set of Proba-NK (AgroDiagnostica, Russia). MDMV and BSMV are RNA viruses, and therefore PCR diagnostics is carried out in two stages — reverse transcription and PCR itself. A set of reagents “OT-1” (Syntol, Russia) was used for reverse transcription, following the manufacturer's recommendations. To perform RT-PCR in a single-stage format, MMLV revertase from the same set was added to a test tube with a reaction mixture for PCR, and changes to the thermal cyclic regime were made. A master mix 5X MasCFETaqMIX-0025 (Dialat, Russia) was used for PCR.

Reference virus isolates from DSMZ collection (Germany) were used as control samples: MDMV PC-0802, SCMV PC-0731 and positive controls manufactured by Loewe (Germany): BSMV 07004 PC, BaYDV 07005 PC, BaMMV 07006 PC, BMV 07016 PC, WSMV 07048 PC, WDV 07082 PC, BStMV 07123 PC, WSSMV 07171 PC.

To diagnose MDMV, the applicability of the following primer pairs was evaluated: MCPF/MCPF [5], HCF/HCR [6], MDMVGen8151F/MDMVGen9391R [7], and MDMV-F1/MDMV-R1 [8].

Primers 5'BSMV1a/3'BSMV4a were selected for BSMV diagnosis [6]. Since virus strains differ in the number of genome segments, the site contained in all described strains was selected as a marker sequence. The selected pair of primers are complementary to the α-segment of Barley stripe mosaic virus genome.

Results

According to evaluation results, a pair of MCPF/MCPF primers showed very low specificity — amplification led to the formation of products of different lengths when using not only MDMV but also Maize chlorotic mottle virus

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and Sugarcane mosaic virus as a genome matrix. When using HCF/HCR and MDMVGen8151F/MDMVGen9391R primers according to the conditions described in the literature, no PCR products were observed on electropherograms.

When using MDMV-F1/MDMV-R1 primers, one PCR product of the expected length is formed. At the annealing temperature given in the literature data [8], a PCR product was formed for both MDMV virus and closely related SCMV. To increase the specificity, the annealing temperature of the primers was selected. The formation of a PCR product of the expected length only for the target virus was achieved at an annealing temperature of 62 °C. The opportunity of diagnosing Maize dwarf mosaic virus with MDMV-F1/MDMV-R1 primers was demonstrated during the work both with 1-stage and 2-stage PCR with reverse transcription (RT-PCR) (Fig. 1).

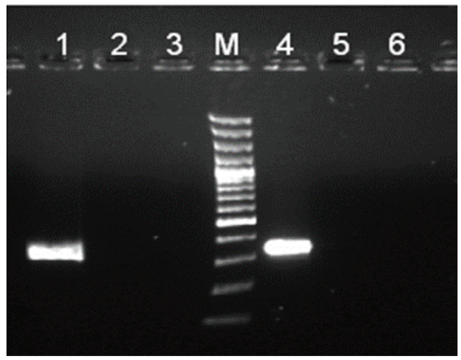

FIGURE 1. MDMV detection by 1-stage (1–3 samples) and 2-stage (4–6 samples) RT-PCR using MDMV-F1/MDMV-R1 primers. 1, 4 – MDMV PV-0802 isolate (DSMZ); 2, 5 – SCMV PV-0731 isolate (DSMZ); 3, 6 – negative control (water)

The operability of the diagnostic test system with these primers was confirmed on samples of corn seeds.

When testing five samples of corn seeds of different origins, a specific amplification product of the expected length was obtained for a sample of corn seeds from the Kabardino-Balkarian Republic. A similar amplification product was obtained for a positive control sample (Fig. 2).

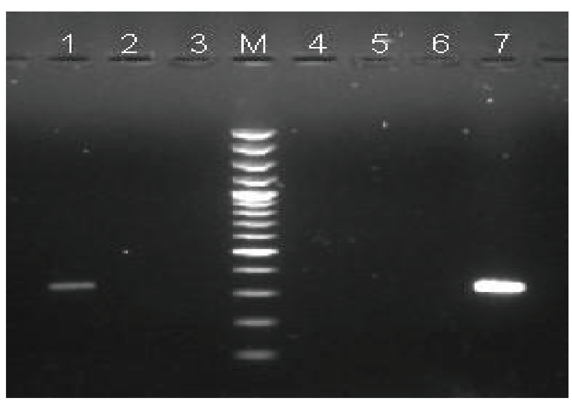

FIGURE 2. MDMV detection by 1-stage RT-PCR in corn seed samples. 1 – corn seeds, Mashuk 171MV F1, the Kabardino-Balkarian Republic, 2 – corn seeds, Krasnodar 194MV F1, Krasnodar Krai, 3 – corn seeds, Tirnavia F1, France, 4 – corn seeds, Ross 199MV F1, Moscow region, 5 – corn seeds, Rostov region, 6 – negative control, 7 – positive control – MDMV PV-0802 isolate (DSMZ)

The obtained amplification products of the Kabardino-Balkarian Republic sample and MDMV PV-0802 (DSMZ) isolate were sequenced. Nucleotide sequence of these products corresponds to the sequence of viral coat protein gene sections of various MDMV virus isolates annotated in NCBI database, which indicates the validity of the obtained results.

Primers 5'BSMV1a/3'BSMV4a [9] showed high specificity in the diagnosis of BSMV in single-stage RT-PCR. During such RT-PCR with BSMV isolates, Barley yellow dwarf virus, Barley mild mosaic virus, Brome mosaic

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virus, Wheat streak mosaic virus, Wheat dwarf virus, Brome streak virus, and Wheat spindle streak mosaic virus, the amplification product of the expected length (180 bps) was synthesized only for target virus.

These primers were tested for the diagnosis of Barley stripe mosaic virus in corn and wheat seeds (Fig. 3). All corn samples showed the absence of the virus in the seeds. In one sample of spring wheat (Altai cultivar) from Moscow region, the presence of the virus was shown.

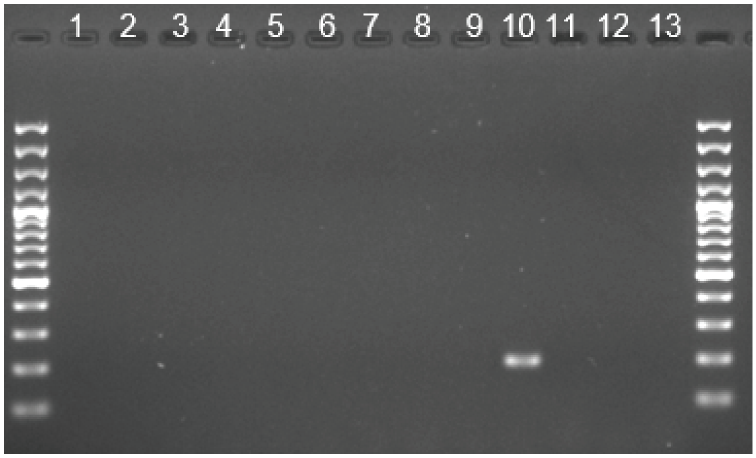

FIGURE 3. BSMV detection by 1-stage RT-PCR in samples of corn seeds (1–5) and wheat (7–12). 6 and 13 – OK. 1 – corn seeds, Mashuk 171MV F1, the Kabardino-Balkarian Republic, 2 – corn seeds, Krasnodar 194MV F1, Krasnodar Krai, 3 – corn seeds, Tirnavia F1, France, 4 – corn seeds, Ross 199MV F1, Moscow region,– corn seeds, Rostov region, 6 – negative control, 7 – spring wheat Kumus, 8 – spring wheat Faraon, 9 – spring wheat Moskovskaya 35, 10 – spring wheat Altai 99, 11 and 12 – spring wheat SOLO, 13 – negative control

Thus, the diagnostic test system has shown its applicability for detecting Barley stripe mosaic virus in grain.

Discussion

According to the test results, a pair of MCPF/MCPF primers showed very low specificity, and, therefore, it is not recommended for use in diagnostics. HCF/HCR and MDMVGen8151F/MDMVGen9391R primers did not amplify any PCR products, and, therefore, their use is not recommended as well.

When performing PCR with MDMV-F1/MDMV-R1 primers, one PCR product of the expected length is formed, but a PCR product is formed for both MDMV and closely related SCMV, which required an increase in the annealing temperature of the primers to 62 °C. The use of MDMV-F1/MDMV-R1 primers both in 1-stage and 2-stage PCR with reverse transcription (RT-PCR) allows us to obtain good PCR results, which was confirmed on samples of corn seeds.

Nucleotide sequence of the obtained amplification products of the Kabardino-Balkarian Republic sample and MDMV PV-0802 isolate (DSMZ) after sequencing corresponded to the sequence of viral coat protein gene sections of various MDMV virus isolates annotated in NCBI database, which indicates the validity of the obtained results.

Primers 5'BSMV1a/3'BSMV4a [9] showed high specificity in the diagnosis of BSMV in single-stage RT-PCR. The amplification product of the expected length (180 bps) was synthesized only for the target virus.

All samples of corn seeds were free of virus. In one sample of spring wheat (Altai cultivar) from Moscow region, the presence of the virus was shown.

To confirm infection with this virus, nucleotide sequence of PCR product was determined. The resulting sequence corresponded to the sequence of BSMV genome section, and, therefore, this test system can be recommended for detecting this virus in grain.

Conclusion

Based on the analysis of the main phytosanitary requirements of the importing countries, the most important Maize dwarf mosaic virus and Barley stripe mosaic virus were selected as creating risks of banning the import of a significant amount of grain products from the Russian Federation. The tested primers and PCR kits have shown the opportunity of using them for the diagnostics of MDMV and BSMV. Optimal reagents and conditions of their application for the diagnostics of the studied viruses in grain samples of host plants were selected.

Acknowledgments

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Features of the Formation of Secondary Resources Processing of Sugar Industry in the Kyrgyz Republic

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Abstract. The article reveals the research conducted by the authors in determining the features of the formation of secondary resources processing of sugar industry in the Kyrgyz Republic by development stages. The justification of the main stages of sugar industry development and processing of its secondary resources is carried out by identifying important reforms that have affected the economic development of the state and vice versa. When carrying out a comprehensive assessment of the features of the formation of sugar industry development and the processing of secondary resources of the sugar industry, the main production indicators were used such as planted area, yield, sugar production from beets, the volume of formation of secondary resources from sugar beet (transport and washing waste and tops, pulp, syrup and molasses, and filtration precipitation). A positive dynamics of the development of the sugar industry is observed in all parameters. Nevertheless, sugar factories do not take into account the formation of such sugar production waste as transport and washing waste and filtration precipitation, despite a sufficient proportion of the volume of sugar beet 10-12%. Consequently, an increase in the large-tonnage waste of sugar production, which needs accounting, control, rational further use in the national economy of the state occurs. In this regard, the priority areas of sustainable development of sugar beet production, to implement import substitution, maybe the development and implementation of resource-saving technologies, including the use of secondary resources.

INTRODUCTION

The sugar industry is one of the promising directions of socio-economic development of the state, ensuring food security of the country and contributing to the development of agricultural production [1, 2].

Sugar beet is the most valuable technical crop, which makes it possible to produce a food product necessary for humans from its root crops – granulated sugar [3], in turn, to obtain many valuable products from the secondary resources of the sugar industry to develop livestock breeding, crop production [4], and other strategically important sectors of the economy of the Kyrgyz Republic.

MATERIALS AND METHODS

A systematic approach, a sample survey method, abstract-logical and statistical-economic research methods are applied.

RESULTS

The features of sugar industry development and the processing of secondary resources of sugar production in the Kyrgyz Republic are revealed.

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DISCUSSION

The formation features of the processing of secondary resources of the sugar industry are inextricably linked with the development of sugar production in the Kyrgyz Republic, which is characterized by four main stages, where the beginning of each of them is determined by important reforms, and its impact on economic development and vice versa is implanted (Fig. 1):

- The first stage (1991-1997) is characterized by the transition from the former post-Soviet economic system to a market economy.
- The second stage (1998-2008) is about sugar production development due to the increase in world prices and demand for sugar, which led to modernization of sugar beet production technologies and its processing.
- The third stage (2009-2015) is characterized by the development of a concept for sugar industry development and bringing it to the requirements of the tasks of economic development of the state.
- The fourth stage (2016-now) is characterized by important changes, as in the overall economic development of the country in the conditions of the Customs Union.

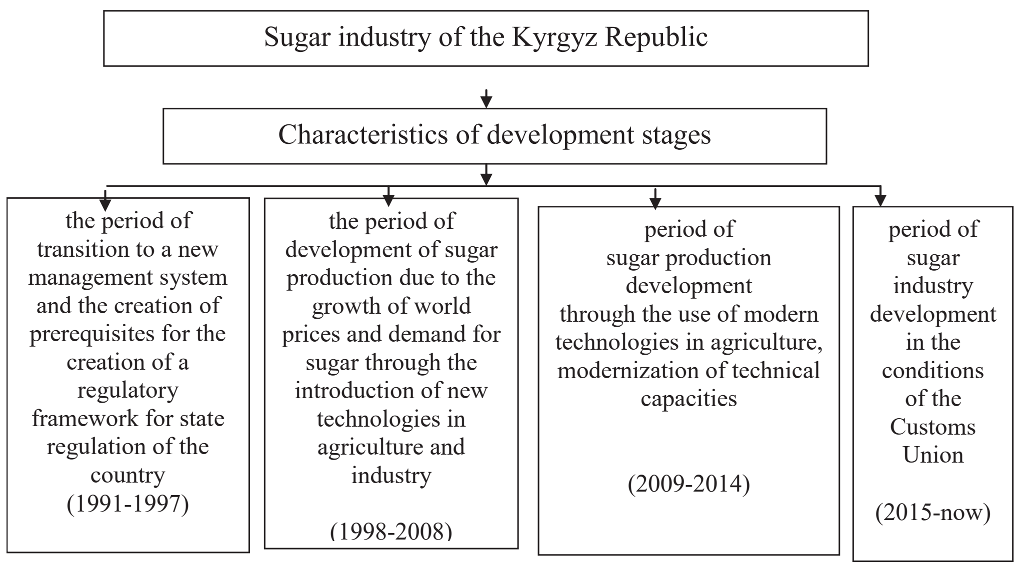

FIGURE 1. The way of sugar production development in the Kyrgyz Republic and its characteristics [5]

Stage 1. In the 80s of the 20th century, the production of sugar beet root crops in the Kyrgyz Republic was suspended due to critical situations that were associated with the massive spread of root rot and beet worms in the fields.

After gaining sovereignty in 1991, the Kyrgyz Republic was made to purchase imported expensive sugar and issue it to the country's population on coupons due to financial and economic difficulties. One of the important decisions was made to resume the production of sugar beet root crops in the country itself. By comparing the main indicators of the development of the sugar subcomplex of the agro-industrial complex of the Kyrgyz Republic, we can observe that the transition to a market economy in this industry was very smooth since the volume of planted area is average compared to previous periods. The main reason for this phenomenon was the fear of farmers of the resumption of root rot occurring at this time.

The volume of planted area grew gradually during the transition period until 1998, starting from 103 hectares in 1990 up to 11.6 thousand hectares, then the level of the sugar industry development began to rise sharply (the maximum growth rate was 338%).

Stage 2. Due to the appreciation of the US dollar on the world market [6] and a significant increase in prices for white sugar, a situation that is significantly beneficial for beet producers has arisen in the Kyrgyz Republic. As a result, the planted area of sugar beet in 1998 increased sharply to 22.4 thousand hectares and in 2003 reached a maximum volume of 33.2 thousand hectares. At the same time, beet growers who wanted to extract maximum profit

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from the production of sugar beet bought high-yielding seeds of European hybrids and precision seeders for their sowing independently. This made it possible to increase the yield of sugar beet root crops by 260.3 dt/ha in 2003 and 242.6 dt/ha in 2004, as well as the gross harvest of root crops, had record figures in these periods — 812.3 TMT and 642.4 TMT respectively.

Thus, based on the history of sugar industry development, the influence of the global sugar market is observed [7], which allowed increasing the socio-economic level of the economic development of the Kyrgyz Republic by increasing the gross harvest (353%) and yield (120%) of sugar beet compared to the previous period (Table 1).

TABLE 1. Dynamics of gross harvest and yield of sugar beet of the Kyrgyz Republic [8]

Name of indicators/ Stages of sugar industry development	Stage 1 (1990-1997)	Stage 2 (1998-2008)	Stage 3 (2009-2014)	Stage 4 (2015-now)
Total gross sugar beet harvest (TMT)	123.2	434.8	117.6	593.4
Growth rate compared to the previous period, %	-	353	27	504.6
Sugar beet yield (dt/ha)	167.2	201.3	140.8	468.3
Growth rate compared to the previous period, %	-	120	70	333
Value of indicators	Average	Near to maximum	Minimum	Maximum

However, only two years later, when the prices of white sugar stabilized and decreased, the interest in sugar beet production among domestic beet growers fell again, as a result of which the planted area of sugar beet farming has already been only 14.5 thousand hectares in 2005, and in 2008 its cultivation was over completely.

Such negative results have arisen for various reasons. For example, after the fall of the Soviet Union, the machine and tractor fleet of beet producers as a whole was not refreshed. Due to this fact, agricultural machinery, which is significantly important for the cultivation of sugar beet, is outdated, especially seeders that sow a large dose of seeds, the use of outdated equipment [9], without providing high-quality soil preparation for sowing, as well as the timely organization of sowing, ultimately it was not possible to plant sugar beet, which is why most domestic beet growers could not receive necessary shoots of sugar beet.

Taking into account the dynamics of previous years, the yield of sugar beet obtained from German seeds has significantly exceeded the yield of beet grown from locally produced seeds. High-quality seeds allow farmers to increase the yield of sugar beet, which for the plant is expressed in additional beetroot, respectively, in additional finished products and income. So, from 2009 to nowadays, the yield of sugar beet has increased from zero to 475 dt/ha.

Stage 3. At the same time, development of the sugar industry depends on development of sugar beet production. Since, in the period from 1995 to 2007, the production of sugar of domestic production decreased significantly, and in 2008 it has stopped altogether. Then the most difficult period of the sugar industry recovery has begun due to financial problems and lack of funding from investors, which belongs to the third stage (2009-2014).

As one of the significant measures for sugar production development and the interest of beet producers to increase the planted area for sugar beet cultivation for 2011 and 2013, the plant has purchased in the West Germany elite seeds of sugar beet in 2011 – for the amount of 7.458 million soms including the German company “KVS” in the amount of 2,000 sowing units to the amount of 96 thousand euros, in 2012 – for the amount of 22.1 million soms, from the company “KVS” in the amount of 9,000 sowing units to the amount of 444 thousand euros. Seeds were distributed to farmers with deferred payment, in the form of commodity loans, for the harvest of 2011-2013.

To significantly improve the welfare of several thousand farmers of the Kyrgyz Republic, and their grown products (sugar beet) were in demand, the plant employees improve the conditions of contracts for the reception and processing of sugar beet. According to numerous requests of beet producers, since 2011, the plant began to accept sugar beets from them without taking into account digestion (sugar content). In this connection, the farmers confidence in the plant has grown.

Stage 4. Only in recent years, domestic producers of sugar beet and sugar have begun to pay great attention to the production of sugar and sugar beet. A comparative analysis of sugar production by stages of development shows that the introduction of modern technologies leads to high rates of productivity growth. In particular, at the fourth stage of the development of the sugar industry, the average value of sugar productivity for only three seasons reached the maximum level (64.8 mt/year).

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The main key indicators of sugar production development are the use of modern technologies in agriculture, imported seeds, and technical capacities in the production of sugar beet [10], which provided the maximum level of their yield in 2018 – 773 TMT in 16 thousand hectares of planted area, which shows the level of yield (475 mt/ha of sugar beet yield) compared to 2009, due to which the efficiency of sugar beet production increased 2.5 times, which led to an increase in sugar production respectively.

Consequently, the sugar industry directly has a close connection with agriculture development. One of the important directions for reducing import dependence and providing at least half of the needs of the domestic market with sugar is to expand the planted area [11] for sugar beet to develop the sugar industry of the country.

According to scientists, practice shows the effectiveness of state regulation of the most important segments of the food market, in particular, the sugar market [12], where, with the help of state influence, it has been possible over the past few years to implement an import substitution policy and somewhat reduce the import dependence of the domestic market on sugar consumption.

The development of the sugar industry of the Kyrgyz Republic in 2013-2017, the volume of sugar beet taken and their further processing had multidirectional trends of change: both a sharp increase and a decrease. In 2017, sugar beet processing amounted to 641.8 TMT, the growth rate compared to 2013 was 388%. Such positive dynamics is observed in the formation of secondary resources of the sugar industry, such as pulp and syrup-molasses. In 2017, a large proportion in the actual output volume of sugar production waste from the accepted weight of sugar beet is pulp – 73%, molasses – 3.1%. The share of secondary resources of the sugar industry from the volume of sugar beet taken in the form of pulp for 2013-2017 varies from 67-73% and molasses – for 3-4% (Fig. 2.).

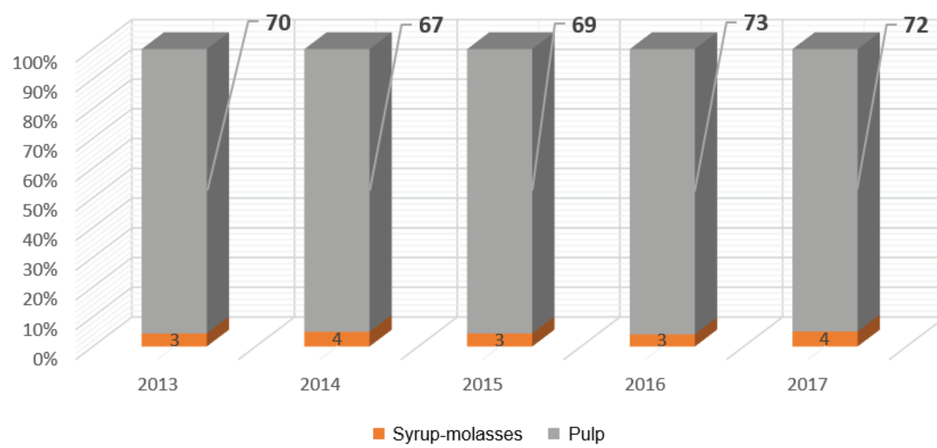

FIGURE 2. The specific weight of the formation of secondary resources of sugar production in the Kyrgyz Republic for 2013-2017 [13]

During sugar beet processing, at the initial stages of cleaning sugar beet from tails and stones, transport and washing waste is formed, as well as filtration precipitation is formed in the filtration processes [14].

According to Fig. 2, it is possible to obtain average coefficients of formation of secondary resources on the example of production indicators of sugar factories by percentage ratios to the total volume of taken sugar beet [15]:

- the formation of transport and washing waste – 12%;
- formation of pulp – 71%;
- formation of syrup-molasses – 4%;
- formation of filtration precipitation – 13%

CONCLUSION

A comparative analysis of sugar production by stages of development shows that the introduction of modern technologies leads to high rates of productivity growth. Currently, sugar factories do their best to have a significant supply of sugar beet grown in the Kyrgyz Republic since the production of white sugar from local raw materials – sugar beet – will make it possible for the republic not to depend on sugar price spikes on the world market and ensure food security of the republic, increase employment. Consequently, there is a great need for the use of low-

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waste and non-waste technologies in the sugar industry, taking into account the greening of production and increasing the efficiency of processing waste from the sugar industry in general.

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Assessing the Cultivation of Organic Green Beans in Western Siberia

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Abstract. Organic agriculture is a new, developing trend in Russia. For this reason, a complex study of the best domestic varieties of green beans is of crucial importance. In particular, research should regard the most effective ways for the realization of the adaptive and bioenergetic potential of this crop as well as the elaboration of variety-specific agrotechnologies for its cultivation within the framework of organic farming. The trials were conducted on the experimental plot for selective crop rotation at the experimental field of the Laboratory of Plant Breeding and Seed Production of Field Crops named after S.I. Leontev of Omsk State Agrarian University. Based on the technical map and the calculation of the direct costs, the results of the research allowed us to determine the economic parameters and economic efficiency of the cultivation of green beans in relation to variety-specific characteristics. The calculations showed that the volume of the assumed net revenue per surface unit changes depending on the variety when green beans are cultivated for seeds (organic products). Thus, the revenue obtained from beans of the Pamyati Ryzhkovoi variety amounted to 110,002.07 rubles, from beans of the Marussya variety to 73,859.40 rubles; these data exceed the Zoloto Sibiri variety, here considered as the control sample, by 65,525.36 and 29,382.69 rubles, respectively. The cost-benefit ratio amounted to 39.9-93.6 %.

INTRODUCTION

A great advantage of agricultural production is that, under any global economic conditions, there will always be a high demand for food commodities. Yet how useful can be these commodities, if they are cultivated using a huge amount of fertilizers and pesticides? At present, people are suffering not only due to a lack of trace elements and minerals in their food but also due to the uncontrolled effects of chemicals on the environment. People's immunity is massively decreasing and this results in outbreaks of different epidemiological diseases. The main issue, however, is no longer the unavailability of food, but the quality of foodstuff. Thus, the shift towards quality is an essential, topical, pivotal turnover in agriculture. Centuries of practice have stabilized conventional agrotechnologies which are now well-known. On the other hand, organic farming is a new trend that has just begun developing in Russia. Federal Law No. 280-FZ on organic farming was enacted in January 2020 [1, 2]. To meet the current demand for domestic organic production, experts say, the number of producers should be increased yearly by 200. The supporters of healthy nutrition, promoting and consuming natural environmentally friendly products, are constantly increasing. Yet the shift from industrial to organic production is a long-lasting process [3, 4, 5].

The foundation of organic farming is the fertility of the soil. In this regard, crop rotation plays a particularly important role. Legumes occupy a crucial position in crop rotation as the main providers of nitrogen to the ecosystem during the cultivation of organic commodities [6, 7]. To broaden the range and cultivation area of legumes in Siberia, new adapted varieties must be bred and spread. Both dry and green beans are of crucial interest since the implementation of rational nutrition requires increasing the nutritional value of commodities while reducing their production costs [8, 9].

The yield of legumes, e.g. of green beans, is twice or three times as much as that of cereals, thus providing agricultural producers with further economic opportunities [9, 10]. The plant residues of legumes can be used as feed for livestock, increasing the concentration of nitrogen in their ration, improving their health conditions, and boosting their growth. In addition, legumes have extraordinary genetic diversity, which gives them a great potential for adaptation to climate change. As a result, agricultural producers can select certain varieties to fully adapt their production to the changing climatic conditions [8, 11].

Thereby, the following research topics are of crucial importance: the in-depth study of the best domestic varieties of green beans; the search for the most efficient way to fulfill their adaptive and bioenergetic potential; and the elaboration of agrotechnologies when cultivating them in organic farming.

Overall, the development of an organic food market is a multifaceted issue that needs a comprehensive approach to elaborate efficient sectoral strategies. Thus, research in this field is of crucial importance [12, 13, 14].

Materials and Methods

The research included trials conducted in 2019-2021 at Omsk State Agrarian University on the experimental field of the Laboratory of Plant Breeding and Seed Production of Field Crops named after S.I. Leontev. The experiments involved three varieties of green beans bred at the University: Pamyati Ryzhkovoi, Marussya, and Zoloto Sibiri, Fig. 1. The last variety served as the control sample.

The crops were mechanically sown using a tractor-mounted selective seed drill SSN-7. The depth of seed placement amounted to 5-6 cm. The area of the plot comprised 0.25 ha. The seed rate varied between 100 and 110 kg/ha depending on the variety and the thousand kernel weight. The forecrop was wheat.

a) b) c)

FIGURE 1. Green bean varieties bred at Omsk State Agrarian University: a. Pamyati Ryzhkovoi, b. Zoloto Sibiri, and c. Marussya (Compiled by the authors)

The crop tending included two harrowings (before and after shooting) and two manual weedings.

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All the observations, reports, and analyses were executed following the Methodological guidelines for the study of leguminous crop collections (All-Russian Institute of Plant Industry, 1975), the Methodological guidelines for the application of the classifier for the genus Phaseolus L. (bean) (All-Russian Institute of Plant Industry, 1980), and the Methodological guidelines for the study of samples from the global collection of beans (All-Russian Institute of Plant Industry, 1987). The resistance of the samples to anthracnose in field conditions was determined during the phase of biological ripeness of seeds using an infestation scale according to the classifier (All-Russian Institute of Plant Industry, 1984). The plants (30 per variety) were manually harvested during the phase of full ripeness to determine their yield structure. The meteorological conditions of the vegetation periods were assessed using data from the Omsk Meteorological Station. The experimental data were elaborated using Microsoft Excel and SPSS (version PASW Statistics 20.0).

The seeds are certified as organic according to Federal Law No. 280-FZ "On organic production and on the amendment to specific legislative acts of the Russian Federation", enforced in January 2020, and Interstate Standard GOST 33980-2013 "Organic commodity production".

Results

Based on the technical map and calculation of direct costs, the research allowed us to determine the economic parameters and the economic efficiency of green bean cultivation depending on variety-specific characteristics.

Table 1 shows the calculation of the economic efficiency of green bean varieties cultivated for (organic) seed productions.

TABLE 1. Calculation of the economic efficiency of green bean varieties cultivated for seed production using organic agrotechnologies, 2021

Parameter	Variety		
	Zoloto Sibiri, control sample	Marussya	Pamyati Ryzhkovoi
Yield, t/ha	2.40	2.90	3.50
Direct costs, rub./ha	111,523.29	114,640.60	117,497.93
Prime cost of seeds, rub./t	46,468.04	39,531.24	33,570.84
Selling price of seeds, rub./t	65,000.00	65,000.00	65,000.00
Sales revenue of seeds, rub./ha	156,000.00	188,500.00	227,500.00
Revenue, rub./ha	44,476.71	73,859.40	110,002.07
Profitability, %	39.9	64.4	93.6

The results of the research have shown that the yield of the Pamyati Ryzhkovoi and Marussya varieties exceeds the Zoloto Sibiri control sample by 1.1 and 0.5 t/ha, respectively.

The direct costs, and subsequently the prime cost of the cultivated bean varieties ranged from 33,570.84 to 46,468.04 rub./t. The main voices in the prime cost structure were expenses relating to labor remuneration, seeds, and the maintenance of the basic assets.

The calculations have shown that the amount of net revenue per surface unit oscillated depending on the variety when cultivating green beans for (organic) seeds. The revenue from selling Pamyati Ryzhkovoi beans amounted to 110,002.07 rub., that from Marussya beans to 73,859.40 rub., thus exceeding the Zoloto Sibiri control sample by 65,525.36 and 29,382.69 rub., respectively. Their profitability ranged between 39.9 and 93.6 %.

Thus, the cultivation of green beans for the production of organic seeds is economically viable, profitable, and remunerative in the Omsk Region. With a yield of 2.4-3.5 t/ha and a selling price of 6,5000 rub./t, the profitability can reach 93.6 %.

A similar level of profitability of organic commodities (seeds) is possible only when you choose the most suitable varieties for specific soil and climatic conditions and depending on the technological possibilities for their cultivation.

For both the private sector and agricultural enterprises, Marussya (with ultrathin pods) and Pamyati Ryzhkovoi are the most suitable green bean varieties for cultivation in the southern forest-steppe of Western Siberia. They are ideal for the local soil and climatic conditions, being bush-shaped with a high first pod insertion (this allows mechanical harvesting), high yield, and aptness to canning and freezing.

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Discussion

Issues related to the (practical) biological intensification of vegetable growing (plant farming) can be faced by enriching the crop rotation with leguminous crops. Legumes ensure a significant drop in the technogenic impact on the environment. In addition, the agrotechnical value of beans in the crop rotation structure allows to "treat" the soil and provides high-protein commodities [15].

Omsk State Agrarian University has rich experience in the production of organic seeds of common bean varieties bred at the University itself. These varieties are recorded in the register of the Ministry of Agriculture under certificate No. OCRU.2109.C0023 dated 28 September 2021, and classified as "production of certified organic commodities".

Agricultural production is of crucial importance for the population of Russia. The government support and the WTO arrangements have created the ideal conditions for a comprehensive production, which includes farmlands, crops, livestock, and final consumers as its participants [2, 6]. It should be noted that the new economic conditions have radically changed the attitude towards crop cultivation technologies, crop tending during the vegetation period, harvesting and transport, storage, and yield commercialization in most collective and peasant farms. Production methods are strictly evaluated according to expenditures: the costs of seeds, combustibles, pesticides, etc. [10].

The production efficiency of plant commodities depends directly on the chosen crop, variety, and production technology. In the southern forest-steppe of Western Siberia, the broadening of legume production (in particular, beans) can increase both soil fertility and the overall efficiency of integrated agricultural production.

However, beans have never been cultivated on large scale in Western Siberia. They were an allotment crop. The present research regards the cultivation of green bean varieties using organic farming technologies in Western Siberia. It has shown that the sales revenue of 100 kg of green bean pods is twice as much as the expenditures related to its productions. Thus, the organic cultivation of beans is most promising in horticultural partnerships and peasant farms.

The main products of beans – seeds and green pods – are easily transported and expensive. Generally, Russia imports them frozen from Poland. The cost of plant-based raw materials is constantly changing. For this reason, using the current economic methods, giving an objective evaluation of the cultivation efficiency of a specific crop using one or another production technology is a complicated task.

A flaw in organic vegetable farming is the prime cost of commodities, which is significantly higher if compared to industrial production [16, 17]. In addition, the shift towards organic production in agriculture might decrease yield by 10-50 %. For the most part, it certainly depends on the specific crop and the previous production technologies, yet the yield drop is quite remarkable in any case. Apart from a decrease in the production volume, an increase in labor-related expenses influences the profitability, since most operations have to be executed manually, thus increasing the workload of the production process [4, 18, 19].

The obtained results can improve the efficiency of the organic seed production of legumes. In addition, they can foster the broadening of the range of early ripening vegetable crops in Siberia, just when the population is lacking this type of product. Another important priority is also maintaining the ecological balance in the area. The conducted research is of practical importance: it can show agricultural producers what organic farming and organic commodities are. It can also allow them to see how an organic production of legumes is grown and produced (using green beans as an example) on the Experimental Field of Omsk State Agrarian University.

The obtained organic sowing material, i.e. green beans bred at Omsk State Agrarian University, will be sold in local shops and become available for agricultural enterprises and peasant farms. This is part of the implementation of the national import-substitution project and one of the tasks included in the guidelines food security of Russia: "to reach a share of domestic seeds not inferior to 75 %".

Conclusion

As a result, the current research can help solve the problem of producing specific sowing material for the cultivation of green beans for Western Siberia and also organic commodities (green beans) for the food processing industry. Other benefits include the improvement of the farmlands and ecosystems, as environmentally friendly commodities are produced and new work positions are created in rural areas. This can fasten the process of

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broadening and promoting diversification in the agricultural sector by introducing legumes in the crop rotation system. This can also increase the sustainable competitiveness of organic agricultural commodities in Western Siberia.

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The Use of Probiotic "Yarosil" in Poultry Farming

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Abstract. The article discusses the indicators of growth intensity, biochemical analyzes of blood and droppings, economic efficiency of the use of the probiotic preparation "Yarosil" in dosages of 0.2 and 0.6 ml / kg of live weight on broiler chickens of the Ross 308 cross. The differences in the average daily growth of chickens in the experimental groups were revealed: on average, over the period of the experiment, this indicator was 41.5 g and 40 g, which is 5.3 and 1.5% higher than that of the analogs from the control group. The biochemical parameters of the blood serum of chickens were studied, their analysis allows us to conclude that the probiotic has a positive effect on the activation of protein synthesis, normalization of liver function and on the enhancement of the immune status of the bird. The article presents calculations of the economic efficiency of the use of this probiotic. Additional income from the conditional realization of the gross increase in live weight of poultry in the experimental group of broilers with a dosage of 0.2 ml / kg of live weight amounted to 190.21 rubles, which is 35.72 rubles / bird. (23.1%) more than in the control.

INTRODUCTION

The study of the symbiotic relationship between the microbiota and the host organism is one of the central sections of ecology. A mature and stable gut microbiota in chicks is important for health and productivity. Bilal M. et al., Studying the role in the early maturation of the intestinal microbiota, found that the abundance of Lactobacillus and Bifidobacterium was higher, while the abundance of Enterobacteriaceae was lower in the probiotic groups. Thus, probiotics effectively helped the caecal microbiota reach a mature configuration at an earlier age [1]. Wang D. et al. studied the effects of *Paenibacillus polymyxa* 10 and *Lactobacillus plantarum* 16 on growth performance and gut health in broilers. While Lac16 was found to improve the initial growth rates (body weight and feed conversion) of broilers, the use of BSC10 only slightly improved the initial growth rates of broilers. The results of transmission electron microscopy showed that both probiotics strengthen the intestinal epithelial barrier. The authors conclude that the investigated formulations, especially Lac16, have the potential to positively influence growth performance and gut health in broilers in the start-up phase [2]. Bhogoju S. et al. selected as probiotic bacteria *Lactobacillus reuteri* and *Streptomyces coelicolor* (*S. coelicolor*). Chicken feed containing a mixture of *L. reuteri* and *S. coelicolor* showed a 2% improvement in body weight gain, a 7% reduction in feed intake and a 6–7% reduction in feed conversion rates [3].

Most often, researchers note a small positive effect of probiotics on growth, a more significant one on health, hematological parameters and bird survival [4-10]. Blood glucose and total protein increased when using a high levels of probiotic in water, while cholesterol and LDL (low-density lipoprotein) concentrations were lower in probiotic at the recommended level [11]. A probiotic given increased the total of red and white blood cells but did not affect lymphocyte [12]. In addition to the health benefits, the utility value of the animals increases [13]. Changes in the qualitative and quantitative composition of the caecal microbiota were less pronounced than in the crop [14]. No interaction was observed for weight gain in the starter, finisher, and overall phases. While, during the starter and finisher phases, weight gain was increased by probiotics [15]. The main feature that separates spore-forming probiotics from the more common lactic acid probiotics is their high resistance to external and internal factors,

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resulting in higher viability in the host and correspondingly, greater efficiency [16]. In high altitude regions and probiotic supplementation has no beneficial effects on production performance of broilers at high altitude. However, probiotic supplementation indicated lesser loss due to mortality of birds [17]. The influence of the probiotic "Yarosil" on the productivity and health status of 308 broiler chickens has not been described in the scientific literature.

Materials and methods

The research aimed to determine the effect of the probiotic "Yarosil" on the growth and development of broiler chickens of the Ross 308 cross.

Three groups were formed, two experimental and one control, 13 animals each. Chickens of the first and second experimental groups, in addition to the main diet, together with drinking water were given probiotic "Yarosil" at a dose of 0.2 ml / kg and 0.6 ml / kg of live weight, respectively. The conditions of keeping, feeding and drinking were the same.

Slaughter was performed by decapitation at the age of 45 days. During slaughter, droppings and blood were taken, samples were sent to the Chance Bio laboratory.

The digital research material was processed using a personal computer, Microsoft Office software package.

Results

The results of weekly control weightings show that the chickens of the experimental groups were characterized by a higher dynamics of growth in live weight (Table 1).

TABLE 1. Dynamics of live weight of broiler chickens of the Ross 308 cross, g

Age, days	Group					
	Control		I experimental		II experimental	
	X±Sx	Cv, %	X±Sx	Cv, %	X±Sx	Cv, %
Day-old chicks	42.1±0.2	3.9	42.0±0.2	4.1	41.3±0.3	4.3
7	143.2±6.4	7.3	150.4±5.2	10.4	147.4±4.7	5.1
14	264.1±14.2	11.0	270.3±15.7	14.3	271.7±5.9	6.4
21	515.6±16.4	13.5	531.0±17.7	13.6	521.7±7.1	5.9
28	966.1±25.1	9.3	978.1±34.4	12.7	977.5±17.6	6.5
35	1421.5±35.6	9.3	1494.2±51.7	12.4	1441.5±26.4	6.7

At the day-old age, chickens of all groups did not have obvious differences in live weight, which indicates the identity of the groups. By the age of 7 days, the superiority of the chickens of the experimental groups began to appear relative to the control, which amounted to 5.0% and 2.9%. By the age of 14 days, this superiority was 2.3 and 2.9%. At the age of 3 weeks, the live weight of the birds receiving the probiotic was slightly higher than in the control group – by 2.9 and 1.2%. Also, a slight difference in live weight was at 28 days of age in favor of the experimental groups – by 1.2%. During the remaining analyzed period, the difference increased, and at 35 days of age, it was 5.1% in experimental group I, and 1.4% in experimental group II. The growth rate is determined by the average daily and relative gains in live weight (Table 2).

Indicators of the intensity of the average daily gain indicate that broiler chickens of the experimental groups were characterized by slightly higher growth vigor throughout the entire growing period. At the same time, the growth rates begin to increase from 21 days of age in all groups. By the age of 35 days, the highest average daily gain was found in the birds of the I experimental group, which was higher than the control values by 13.2%. On average, over the period of the experiment, this indicator of the chickens of the experimental groups was 41.5 g and 40 g, which is 5.3 and 1.5% higher than that of the analogs from the control group. Throughout the experiment, the relative growth rate in all groups was approximately at the same level, in the experimental groups this indicator for the period of the experiment (1–35 days) was 189.1 and 188.9%, which is 0.6 and 0.4% higher than in the control group percentage points, respectively. The safety of chickens in our studies in all groups was 100%.

TABLE 2. Average daily and relative gain in live weight of broiler chickens

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Index	Group					
	Control		I experimental		II experimental	
	Average daily gain, g	Relative growth,%	Average daily gain, g	Relative growth,%	Average daily gain, g	Relative growth,%
Age, days: 7	14.4	109.1	15.5	112.7	15.1	112.5
14	17.2	59.4	17.1	57	17.8	59.3
21	35.9	64.5	37.2	65.1	35.7	63
28	64.3	60.8	63.9	59.3	65.1	60.8
35	65.1	38.1	73.7	41.8	66.3	38.4
Over the period of experience	39.4	188.5	41.5	189.1	40.0	188.9
% to the control group	100	-	105.3	-	101.5	-

Blood most fully reflects the biochemical and physiological processes in the body. The results of biochemical analyzes of broiler chickens from the control and experimental groups showed certain differences in their biochemical status (Table 3).

TABLE 3. Blood test of broiler chickens

Biochemical indicators	Group					
	Control		I experimental		II experimental	
	X±Sx	Cv,%	X±Sx	Cv.%	X±Sx	Cv,%
Total bilirubin, μmol / l	2.47±0.29	16.8	2.13±0.32	21.1	2.37±0.04	2.4
Direct bilirubin, μmol / l	0.17±0.04	34.6	0.1±0.0	-	0.17±0.04	34.6
AST, units / l	399.67±56.72	20.1	356.33±47.04	18.6	418.33±19.82	6.7
ALT, units / l	2.67±0.41	21.6	1.67±0.41	34.6	2.33±0.41	24.7
Ritis coefficient	151.23±12.33	11.5	204.93±40.1	27.6	178.3±32.97	26.2
Creatinine, μmol / l	22.33±0.41	2.6	21.67±1.08	7.1	22.0±1.87	12.0
Total protein, g / l	30.53±1.84	8.5	32.23±1.96	8.6	32.77±3.72	16.0
Albumin, g / l	12.5±0.67	7.6	12.73±1.25	13.8	12.8±0.31	3.4
Glucose, mmol / l	17.57±0.59	4.7	16.2±0.43	3.7	15.33±1.16	10.7
Albumin / globulin, g / l	0.69±0.01	2.2	0.65±0.05	10.7	0.66±0.09	19.8
Globulin, g / l	18.03±1.17	9.0	19.5±0.92	6.7	19.97±3.43	24.2

The introduction of a probiotic into the main diet of poultry in experimental groups I and II affected protein metabolism. In the course of the research, it was found that the total protein in the blood serum in chickens of the I and II experimental groups was insignificantly higher than in the control, by 5.6 and 6.8%, respectively, but remained within the reference values for this species. This may indicate the activation of protein synthesis under the action of the probiotic. With regard to the content of serum protein fractions, differences were noted in both albumin and globulin fractions. The content of albumin in the blood serum of chickens of the I and II experimental groups was higher by

1.8 and 2.4%, respectively, in comparison with the albumin of the control group. The globulin fraction changed more significantly: the content of globulins was higher in experimental group I by 8.2%, in experimental group II – by 10.8% compared to the control. These facts may indicate, firstly, an increase in the assimilation of protein-containing feed components under the influence of the probiotic, and secondly, a higher immune status of the experimental bird.

The glucose content in poultry receiving the probiotic also changed, it was lower for experimental groups I and II by 7.8 and 17.7%, respectively, in comparison with the control, all indicators were within the reference interval (9.9-19,3 mmol / L). A decrease in glucose levels against the background of maintaining high productivity indicators may indicate an increase in energy metabolism in birds of the experimental groups.

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Indicators characterizing metabolic processes in the liver also differed in the control and experimental groups. The content of bilirubin in the I experimental group was less relative to the control by 13.8%, in the II experimental group, this indicator was lower by 4.1%. A similar pattern was found for the activity of the ALT enzyme. It was less relative to the control group in birds of the I and II experimental groups by 37.5 and 17.9%, respectively. As for the value of the activity of AST aminotransferase, this indicator for all groups was higher than the physiological norm. However, if in the bird of the I experimental group it was less than in the control group by 10.8% and practically approached the upper limit of the reference values, then in the bird of the II experimental group the AST activity was higher than that in the control group by 12.7%. Thus, the amount of probiotic that was used in addition to the main diet in experimental group I was the most optimal for the normalization of metabolic processes in the liver under the conditions of this study. In the content of creatinine in the blood serum of poultry of the experimental and control groups, insignificant differences were also found. This indicator was less by 3 and 1.5%, respectively, which can be regarded as a certain positive effect of the probiotic on the metabolism of the muscle tissue of the experimental bird.

Dung analysis is part of a diagnostic study of the digestive system and gastrointestinal tract function. In the chickens of the experimental groups, the droppings had a formalized shape, a specific smell, in the control group, it was unformed with a sour odor. The pH of the litter of chickens in the control group had an average value of 5.33, in chickens of the II experimental group – 5.83 (higher by 9.4%). Poultry of all groups lack bilirubin, blood in droppings, connective tissue and muscle fibers, soaps, cellular elements, and extracellular starch. In the control group, the chickens have mucus in the droppings, as well as in the II experimental group, digestible fiber, intracellular starch, fatty acids. The digestibility of feed in poultry in the control group is poor and satisfactory in the experimental groups. At the same time, indigestible fiber is present in the droppings of all birds. Chickens of this group also have fatty acids.

The best values of the indicators of the clinical analysis of the litter can be regarded as a fact reflecting the beneficial effect of the probiotic on the microflora of the gastrointestinal tract of broilers, which can contribute to a decrease in the level of pathogenic microflora.

Based on the results of the experiment, we calculated the economic efficiency of using the probiotic “Yarosil” in broiler chickens of the Ross 308 cross (Table 4).

From the calculations presented in Table 4, it can be seen that in experimental group I, the introduction of a probiotic into the main diet of poultry at a dose of 0.2 ml / kg of live weight will provide additional income from the conditional realization of the gross increase in live weight by

35.72 rubles / bird. more than in the control group. The introduction of the probiotic “Yarosil” into the main diet of broiler chickens of the Ross 308 cross at a dose of 0.6 ml / kg of live weight also increases the profitability of poultry meat production, but only by 10.46 rubles / head in comparison with the control.

Thus, from an economic point of view, the most reasonable dose of Yarosil probiotic in the basic diet of Ross 308 broilers is 0.2 ml / kg of live weight.

TABLE 4. The economic efficiency of the use of the probiotic “Yarosil” for broiler chickens of the Ross 308 cross, per 1 head

Index	Group		
	Control	**I experimental**	**II experimental**
Live weight, g: - day old chicks	42.1	42.0	41.3
- at the end of the experiment	1987.0	2258.3	2090.0
Gross growth over the period of experience, g	1944.9	2216.3	2048.7
± to control, g	-	271.4	103.8
Feed costs for gross growth, rubles	137.24	141.03	138.78
Consumption dose of the probiotic “Yarosil”, ml / kg of live weight	-	0.2	0.6
The cost of the probiotic “Yarosil” for the period of experience, rubles	-	1.20	3.58
Total costs, rub.	137.24	142.23	142.35
Income from the conditional sale of the gross increase in live weight of broilers, rubles	291.74	332.45	307.31
Additional income, rub.	154.50	190.21	164.95

± to control, rub.	-	35.72	10.46

Discussion

The addition of probiotics to broiler chickens diets, studied by other researchers, usually shows little positive effect on body weight gain in chickens. So, scientists of FBGOU VO Gorsky GAU, when studying the effectiveness of the inclusion of probiotic OLIN in the diet of broiler chickens of the Ross 308 cross, obtained a superiority in live weight of chickens from the experimental group over the control group by 2.3% [4]. The experiment carried out in the conditions of the Zagorskoe EPH VNITIP on 4 groups of broiler chickens of the Ross 308 cross showed that drinking the probiotic SUB-PRO at a dose of 5 and 10 mg / l of water allows to obtain the live weight of 35-day-old broilers per 3.80 and 4.51% higher than the control bird with a decrease in feed conversion by 3.17 and 3.70%, respectively [5]. Danilova K.A. also received an insignificant difference in the growth of broiler chickens of the Ross 308 cross when using the probiotic Provagen (1%) [6]. In our studies, the difference in live weight at 35 days of age was 5.1% in the I experimental group, and 1.4% in the II experimental group with the control, which is consistent with the data of other researchers. In the biochemical analysis of blood, scientists from the Kuban and Bashkir State Autonomous Institutions established a significant difference in the total protein content in the blood of chickens receiving the probiotic Trilaktokor [7, 8]. In our studies, an increase was also obtained, but it was not reliable [9]. In terms of the content of ALT and AST, neither in the above-mentioned, nor in our study, there were no significant differences between the experimental groups and the control ones. The presence of intracellular starch in the dung of chickens may indicate insufficient digestion in the intestine, a greater amount of it is observed in the control group. The appearance of fatty acids may be associated with impaired intestinal absorption as a result of increased peristalsis or inflammation.

Conclusion

Thus, our studies have shown that drinking the probiotic preparation “Yarosil” had a positive effect on live weight, growth intensity and the relative growth rate of broiler chickens of the Ross 308 cross.

The best results on the influence of the probiotic preparation “Yarosil” on the above indicators were obtained in experimental group I of broiler chickens, where the dose of probiotic introduction into the main diet of poultry was 0.2 ml / kg of live weight. This is also confirmed by the calculation of the economic efficiency of the use of this probiotic. Thus, additional income from the conditional sale of the gross increase in live weight of poultry in this group amounted to 190.21 rubles, which is 35.72 rubles per 1 head (23.1%) more than in the control.

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Modelling the Variability of Milk Fat Content in Cattle

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Abstract. The authors evaluated the significance of paratypic factors in fat variability in the article. The study looked at the role of fixed effects such as: “Calving Season”, “Calving Year”, “End of Lactation Season”, “End of Lactation Year” and their interacting factors: “Calving Season : Calving Year”, “Calving Season : End of Lactation Year”, “Calving Season : End of Lactation Season”, “Calving Year : End of Lactation Year”. The study used data from primary zootechnical accounting of Holstein cattle (n = 192668) from 2000 to 2020. The authors used linear mixed regression models and appropriate statistical methods and criteria to assess the role of paratypic and genetic factors. The following were selected as random effects: father, age of fertile insemination and animal identification data. The influence of the fixed factors of the prospective mathematical model was evaluated using an analysis of variance. But beforehand, the authors identified different combinations with adjustment for the proportion of random contribution. The grant levels of the estimated factors to the variability of the dependent trait were determined. The authors observed high covariation between predicted and observed fat content ($r = 0.814$; $p < 0.001$). A relatively high coefficient of determination (R2 = 0.662) was observed for the test sample. In this case, only phenotypic data were considered in the example when constructing the model. Application of the resulting model to other subpopulations may require additional correction factors as part of regional or federal breeding value index programs.

Introduction

A fundamental task of most research in dairy farming is to analyze the role of fixed and random factors in the variability of economically valuable traits [1-3]. The variability of quantitative traits is interpreted not only by gene expression but also by a multidimensional paratypic component due to their polygenic nature of inheritance [4-6]. Thus, it is essential to determine the genetic component for breeding traits to assess the breeding value of animals [7, 8]. The bias in evaluating features of interest in high-yielding cattle is particularly evident under harsh climate conditions and scanty feeding [9].

Statistical and mathematical modeling can be a solution to the problem. Among the types of modeling presented, multivariate and multiple regression models are significant [10]. The most popular solutions are the linear and non-linear mixed models underlying BLUP [10-13], including the Bayesian approach [14] and GBLUP models [15]. Such tools are highly effective in relatively small and large industrial complexes [16].

Fat content is a selection-relevant polygenic quantitative trait in dairy farming [17]. Also, fat content in milk is one of the essential nutritional elements in both raw and processed form. Thus, prediction of fat level in milk in an ontogenetic aspect seems to be a significant link in obtaining raw dairy milk with parameters close to those

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expected. The study aims to establish the role of fixed factors in the retrospective variability of fat content in milk of lactating cows under industrial complex conditions.

Materials and Methods

The research was conducted under the conditions of a high-tech dairy complex (West Siberia, Russia) using a database on Holstein cattle. The authors evaluated the variability of fat content (%) and milk fat content (kg) (n = 192668) from 2000 to 2020. The dry matter content was determined in a certified laboratory to control milk quality. Primary zootechnical accounting was carried out in the information-automatic system “SELEX”. The authors then exported data from this system to the statistical programming environment “R”, followed by model building and estimation.

Truncation of the original data was done to account for the role of outliers in reducing the accuracy of the estimates obtained. Daughters and their mothers had milk yields of at least 5000 kg. Fat content was limited to 2.7% to 5.2% and milk fat from 100 to 900 kg. The duration of the open days was 45 to 200 days and the dry period was 30 to 80 days.The authors assessed the significance of genetic and paratypic factors using simple and mixed regression models using the lme4, lm and merTools libraries [10, 18]. The fixed effects were “Calving Season” (CS), “Calving Year” (CY), “End of Lactation Season” (ELS), “End of Lactation Year” (ELY) and interacting factors: “Calving Season : Calving Year” (CS : CY), “End of Lactation Season : End of Lactation Year” (ELS : ELY), “Calving Season : End of Lactation Season” (CS : ELS), “Calving Year : End of Lactation Year” (CY : ELY). Random effects such as “Age of the Fruitful Insemination in proper lactation” (AFI.in), “Father” (F) and “Animal Identification Data” (ID) were represented in the set. The role of fixed factors of the prospective mathematical model in the variation of dependent facets was assessed by analysis of variance. The linear mixed model was described by formula 1 [19].

$$y = \mathbf{X}b + \mathbf{Z}a + e \,, \qquad ()$$

where:

$y = n \times 1$ vector of observations; n = number of records;
$b = p \times 1$ vector of fixed observations; p = number of levels for fixed effects;
$a = q \times 1$ vector of random effects; q = number of levels for random effects;
$e = n \times 1$ vector of residual random effects;
$\mathbf{X}$ = a design matrix of order $n \times p$ that relates entries to fixed effects;
$\mathbf{Z}$ = a design matrix of order $n \times q$ that associates entries with random effects.

The authors considered the influence of random factors when constructing a mixed model [10, 19]. These random factors were associated with the matrix **Z** and were represented by the corresponding set of columns $q_i = l_i p_i$. This value of l_i represents the number of levels of the i-th random factor (2).

The number of such columns was related to the dimensionality of the matrix:

$$q = \sum_{i=1}^{k} q_i = \sum_{i=1}^{k} l_i p_i \,. \qquad ()$$

The elements of matrix Z_i was generated using the Kronecker tensor products of blocks **J** and **X** and is related to the random effect (3).

$$Z_i = \left(J_i^{\mathrm{T}} * X_i^{\mathrm{T}}\right) = \left[J_{i1}^{\mathrm{T}} \otimes X_{i1}^{\mathrm{T}} \; J_{i2}^{\mathrm{T}} \otimes X_{i2}^{\mathrm{T}} \;\; \vdots J_{ij}^{\mathrm{T}} \otimes X_{ij}^{\mathrm{T}} \;\right], \qquad ()$$

where * and $\otimes$ are works of Hatry-Rao and Kronecker [20];

J_i^{T} and X_i^{T} are vectors of rows of j-th row J_i and X_i. These rows correspond to the j-th element of the dependent trait. The entire matrix of the random effects model **Z** was constructed from $k \geq 1$ blocks (4)

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$$\mathbf{Z} = \left[Z_1 Z_2 \ \dots \ Z_k \right]. \qquad ()$$

The initial sample was split into two parts: training and test models. The training model is the basis on which an experimental mathematical model is created, used to evaluate the resulting model. The training sample involved 166,766 animal records, and the test sample – 35,762 animal records. Only forms of daughters of bulls with at least seven offspring in the herd were used due to the limitation of some criteria on the size of the populations. Models were compared using the Akaike and Schwartz criteria [21].

Results

Linear mixed models of variability in fat measured as a percentage and in kilograms were constructed (5). The formula includes fixed and random factors as predictors:

$$\text{Fat}_{kg,\%} = \text{CY+ELY+CS+ELS+CS : CY+CY : ELY+CS : ELS+1} \mid \text{ID+1} \mid \text{FFI+1} \mid \text{F}. \qquad ()$$

Forecasting revealed high predictive accuracy in the test sample, where fat in kilograms between predicted and observed values was taken as the dependent variable. The correlation coefficients were respectively: Spearman 0.814±0.005 (R^2 = 0.663), Pearson 0.799±0.005 (R^2 = 0.638) and Kendall 0.624±0.005 (R^2 = 0.389). Kendall's coefficient of correlation indicates the possible presence of outliers compared to the other ones. These outliers may be due to the size of the original data set. The use of phenotypic data alone showed a high proportion of interpretable variance. It is expected that the introduction of SNP data into the model will increase the value of this indicator due to the increased accuracy of the model. Analysis of variance was conducted to assess the contribution of fixed factors to fat variability (Table 1).

TABLE 1. Role of fixed factors in fat variability

Factor	df		SS Effect		MS Effect		F	
	kg	%	kg	%	kg	%	kg	%
CY	20	20	12001297	173.121	600065	8.6560	138.3163	328.8655
ELY	20	20	51388280	22.554	2569414	1.1277	592.2558	42.8436
CS	3	3	3614622	1.064	1204874	0.3545	277.7262	13.4694
ELS	3	3	8606121	0.665	2868707	0.2215	661.2434	8.4154
CY: CS	58	58	2806775	47.862	48393	0.8252	11.1546	31.3515
ELY: ELS	58	58	1581314	11.293	27264	0.1947	6.2844	7.3976
CY: ELY	38	40	5953916	6.687	156682	0.1672	36.1156	6.3519
CS: ELS	9	9	20739389	6.565	2304377	0.7294	531.1640	27.7133

The End of Lactation Year (ELY) and End of Lactation Season (ELS) showed the highest intergroup variability for the model with the dependent trait fat in kilograms. This variability proves the increased contribution of these factors in predicting variability in fat in kilograms and can be explained by changing animal feeding and keeping conditions over 20 years. Fixed factors such as Calving Year (CY) and End of Lactation Year (ELY) contributed most to the percentage fat calculation. It was found that the accuracy in predicting the amount of milk fat (kg) depended on several random factors, among which animal identification information played a key role (Table 2).

TABLE 2. Variability of random effects in the model for the amount of milk fat (kg)

Source of variability	σ^2		σ		σ^2, %		σ, %	
	kg	%	kg	%	kg	%	kg	%
ID	2225.9	0.265	47.18	0.073	28.19	37.65	28.67	26.45
Father	751.3	0.076	27.41	0.039	9.51	10.76	16.66	14.13
AFI.in	581.1	0.008	24.11	0.002	7.36	1.08	14.65	0.72
Residual	4338.4	0.356	65.87	0.162	54.94	50.53	40.03	58.70

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General	7896.7	0.705	164.57	0.276	100	100.00	100.00	100

The results are explained by the correlation in the original data set. And these results are also supported by the variance, and standard deviation fractions of the pooled sources of random effects accounted. In other words, the variance components and standard deviation were 45.1 and 60 for kilograms of fat as well as the balance of variance and standard deviation were 49.5 and 41.3 for fat percent, respectively (Table 2). The reason for this phenomenon are repitability and kinship. And also, the physiological characteristics of the animals, but to a lesser extent, are evident in a high-tech dairy complex with intensive production use. Visualizing the contribution of random predictors allowed us to assess the magnitude and the density of the distribution of the residuals within the factor's levels (Figure 1).

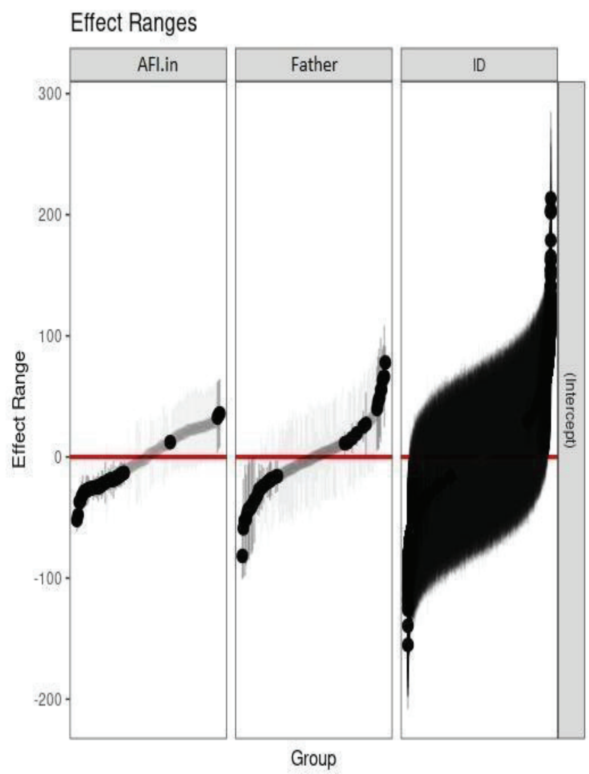

FIGURE 1. "Noise effect" of random factors in the model with the dependent trait quantity of milk fat (kg)

The authors note that the effects included in the model made it possible to significantly reduce the forecasting error and pointed out the need to take them into account when building similar models. Such an approach is actively implemented abroad, but has never found wide application in Russia.

Further estimation in modelling was based on obtaining regression coefficients on fixed predictors (Table 3). The authors provide only t-criterion values with levels greater than or equal to 1.9, given the relative accuracy of estimates of significance levels in models of this type.

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Further estimation in modelling was based on obtaining regression coefficients on fixed predictors (Table 3). The authors provide only t-criterion values with levels greater than or equal to 1.9, given the relative accuracy of estimates of significance levels in models of this type.

TABLE 3: Role of fixed effects in the variability of the amount of milk fat (kg)

FE*	β	βe	t	FE	β	βe	t
CY(2001)	-457.315	156.159	-2.929	CY (2017): CS (summer)	-41.760	10.854	-3.847

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CY(2002)	-456.598	140.803	-3.243	CY (2019): CS (summer)	-35.872	11.317	-3.170
CY(2003)	-318.247	48.892	-6.509	CY (2005): CS (autumn)	-29.299	12.104	-2.421
CY(2004)	-503.858	108.405	-4.648	CY (2006): CS (autumn)	-31.742	12.386	-2.563
CY(2005)	-829.297	132.778	-6.246	CY (2007): CS (autumn)	42.149	12.113	3.480
CY(2006)	-636.517	66.370	-9.590	CY (2010): CS (autumn)	-49.736	12.074	-4.119
CY(2007)	-918.776	105.282	-8.727	CY (2012): CS (autumn)	-37.890	12.226	-3.099
CY(2008)	-1139.592	156.148	-7.298	CY (2013): CS (autumn)	-40.361	12.053	-3.349
CY(2009)	-1014.772	105.379	-9.630	CY (2014): CS (autumn)	-30.238	11.888	-2.544
CY(2010)	-1274.459	155.666	-8.187	CY (2015): CS (autumn)	-39.918	11.774	-3.391
CY(2011)	-956.591	133.826	-7.148	CY (2017): CS (autumn)	-44.302	11.808	-3.752
CY(2012)	-1266.851	172.132	-7.360	CY (2018): CS (autumn)	-34.577	11.725	-2.949
CY(2013)	-1290.397	160.395	-8.045	CY (2019): CS (autumn)	-38.115	11.985	-3.180
CY(2014)	-1315.008	133.211	-9.872	ELY (2018): ELS (winter)	-61.824	28.684	-2.155
CY(2015)	-1581.828	162.115	-9.757	ELY (2001): ELS (summer)	-30.884	11.867	-2.603
CY(2016)	-1703.583	155.270	-10.972	ELY (2002): ELS (summer)	-33.110	9.616	-3.443
CY(2017)	-1714.111	145.139	-11.810	ELY (2003): ELS (summer)	-24.201	8.513	-2.843
CY(2018)	-1931.775	152.122	-12.699	ELY (2006): ELS (summer)	-25.422	8.040	-3.162
CY(2019)	-2048.696	152.062	-13.473	ELY (2007): ELS (summer)	-19.485	8.056	-2.419
CY(2020)	-2139.890	153.625	-13.929	ELY (2009): ELS (summer)	-24.090	8.000	-3.011
ELY(2001)	63.157	25.960	2.433	ELY (2010): ELS (summer)	-28.887	8.086	-3.572
ELY(2002)	147.787	28.345	5.214	ELY (2002): ELS (autumn)	-31.869	10.186	-3.129
ELY(2003)	382.198	54.061	7.070	ELY (2003): ELS (autumn)	-26.534	8.947	-2.966
ELY(2004)	569.029	111.57	5.110	ELY (2004): ELS (autumn)	-17.600	8.378	-2.101
ELY(2005)	895.756	135.255	6.623	ELY (2005): ELS (autumn)	-25.067	8.714	-2.877
ELY(2006)	721.984	70.105	10.299	ELY (2007): ELS (autumn)	-47.154	8.473	-5.565
ELY(2007)	954.409	108.231	8.818	ELY (2009): ELS (autumn)	-22.504	8.316	-2.706
ELY(2008)	1228.631	158.171	7.768	ELY (2010): ELS (autumn)	-25.437	8.343	-3.049
ELY(2009)	1153.888	107.862	10.698	ELY (2011): ELS (autumn)	-30.434	8.175	-3.723
ELY(2010)	1412.410	157.787	8.951	ELY (2017): ELS (autumn)	16.981	7.701	2.205
ELY(2011)	1037.567	136.182	7.619	ELY (2018): ELS (autumn)	-17.163	7.730	-2.220
ELY(2012)	1375.637	173.958	7.908	CY (2001): ELY (2001)	435.166	156.140	2.787

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ELY(2013)	1390.121	162.444	8.558	CY (2001): ELY (2002)	383.218	156.356	2.451
ELY(2014)	1444.337	135.209	10.682	CY (2002): ELY (2002)	358,082	141.183	2.536
ELY(2015)	1717.982	164.009	10.475	CY (2001): ELY (2004)	384.400	127.306	3.019
ELY(2016)	1835.740	157.271	11.672	CY (2003): ELY (2005)	-370.737	123.836	-2.994
ELY(2017)	1889.062	147.003	12.850	CY (2004): ELY (2005)	-300.563	86.289	-3483
ELY(2018)	2167.051	154.089	14.064	CY (2006): ELY (2007)	-208.735	82.002	-2.545
ELY(2019)	2285.912	154.102	14.834	CY (2006): ELY (2008)	-410.737	141.633	-2.900
ELY(2020)	2360.688	153.820	15.347	CY (2007): ELY (2008)	-252.213	115.998	-2.174
CS(winter)	55.885	9.566	5.842	CY (2009): ELY (2010)	-233.084	115.118	-2.025
CS(summer)	-31.295	9.772	-3.203	CY (2009): ELY (2011)	253.242	83.375	3.037
CS(autumn)	26.851	10.983	2.445	CY (2010): ELY (2011)	395.505	83.186	4.754
CS(summer)	89.653	5.999	14.946	CY (2011): ELY (2012)	-279.876	110.409	-2.535
CS(autumn)	137.726	6.485	21.237	CY (2010): ELY (2013)	393.881	124.035	3.176
CY (2006): CS (winter)	-25.963	11.281	-2.301	CY (2014): ELY (2015)	-228.567	92.814	-2.463
CY (2009): CS (winter)	-21.831	11.287	-1.934	CY (2014): ELY (2016)	-197.070	80.310	-2.454
CY (2010): CS (winter)	-23.089	11.119	-2076	CY (2017): ELY (2018)	-171.894	45.918	-3.744
CY (2015): CS (winter)	-29.24	10.667	-2.787	CY (2017): ELY (2019)	-131.522	45.942	-2.863
CY (2019): CS (winter)	-21.988	11.017	-1.996	CY (2018): ELY (2019)	-81.219	9.235	-8.795
CY (2002): CS (summer)	-24.085	11.422	-2.109	CS (winter): ELS (winter)	-14.998	3.225	-4.651
CY (2004): CS (summer)	-21.900	11.323	-1.934	CS (summer): ELS (winter)	122.626	3.969	30.895
CY (2005): CS (summer)	-35.198	11.121	-3.165	CS (autumn): ELS (winter)	29.990	4.338	6.913
CY (2006): CS (summer)	-40.826	11.273	-3.622	CS (winter): ELS (summer)	-18.991	4.690	-4.049
CY (2008): CS (summer)	-29.609	11.252	-2.631	CS (summer): ELS (summer)	-27.298	3.163	-8.631
CY (2013): CS (summer)	-26.552	11.195	-2.372	CS (autumn): ELS (summer)	-112.369	4.495	-24.998
CY (2014): CS (summer)	-22.373	11.103	-2.015	CS (winter): ELS (autumn)	-168.311	4.259	-39.521
CY (2015): CS (summer)	-20.845	10.913	-1.910	CS (autumn): ELS (autumn)	-110.264	5.002	-22.044

*F.E. – fixed effect

The authors note the greatest number of factor's levels with a significant contribution to the variability of the dependent variable among the factors "Calving Year", "End of Lactation Year" and the inter-factor interaction "Calving Year: Calving Season". Their number of gradations was 20 each. A more significant number of gradations also stand out "Calving Year: End of Lactation Year" (19 gradations) and "End of Lactation Year: End of Lactation Season" (18 gradations). At the same time, the inter-factor interaction "Calving Season: End of Lactation Season" had eight gradations, which was half the number of alternations. It is worth noting that the number of gradations with t-criterion values exceeding 3 was highest for the factors "Calving Year" and "End of Lactation Year". These levels were consistent with the data in Table 1 and expressed in the corresponding values of the F-criterion. The authors found the importance of the regression coefficients of the factors "Calving Season" and "End of Lactation Season" to be an exciting feature. The year's season in which the cows started lactation was more significant for cows in a high-tech dairy facility than the season of the end of lactation. The amount of milk fat predominantly tended to change in the summer and winter, regardless of the reference year. And the highest fat amount was achieved in the last three years of the entire observation period, which corresponded to the highest levels of regression coefficients and t-criteria of the random effect "End of Lactation Year" (Table 3).

The characterization of the constructed models was accompanied by the construction of scatter plots of predicted and observed values (Fig. 2). The results showed an acceptable level of approximation with a relatively low proportion of outliers that had no significant effect on the interpretation of the variability in fat in kilograms and a

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low level of approximation in the model with fat as a percentage. Each calving year had a different colour, and the distribution of points in the diagram was unidirectional, which explained the high accuracy of the models.

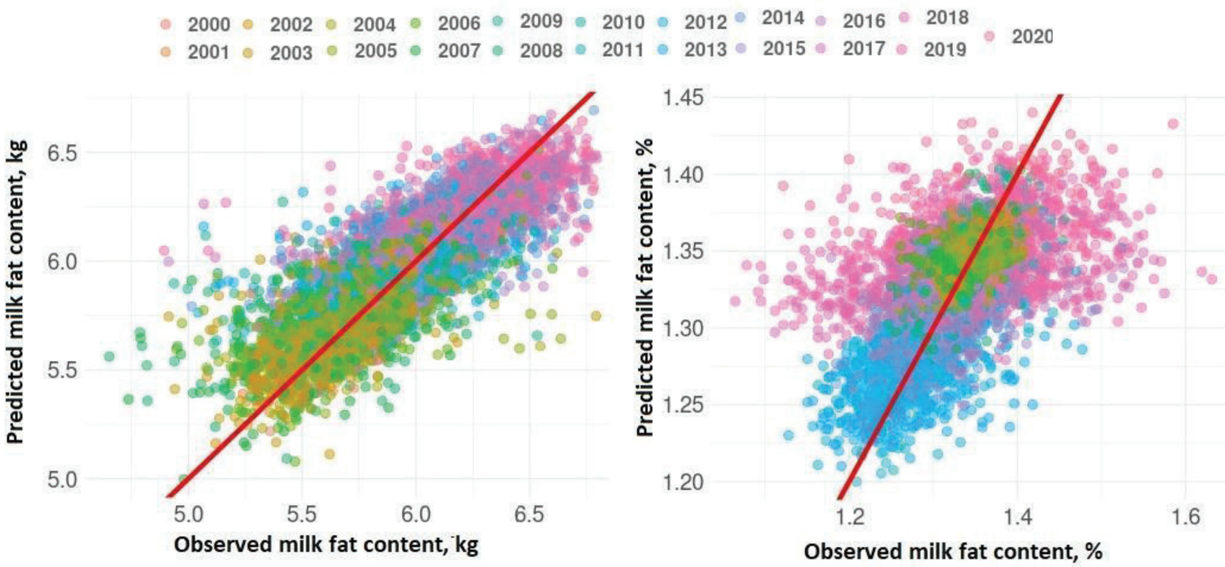
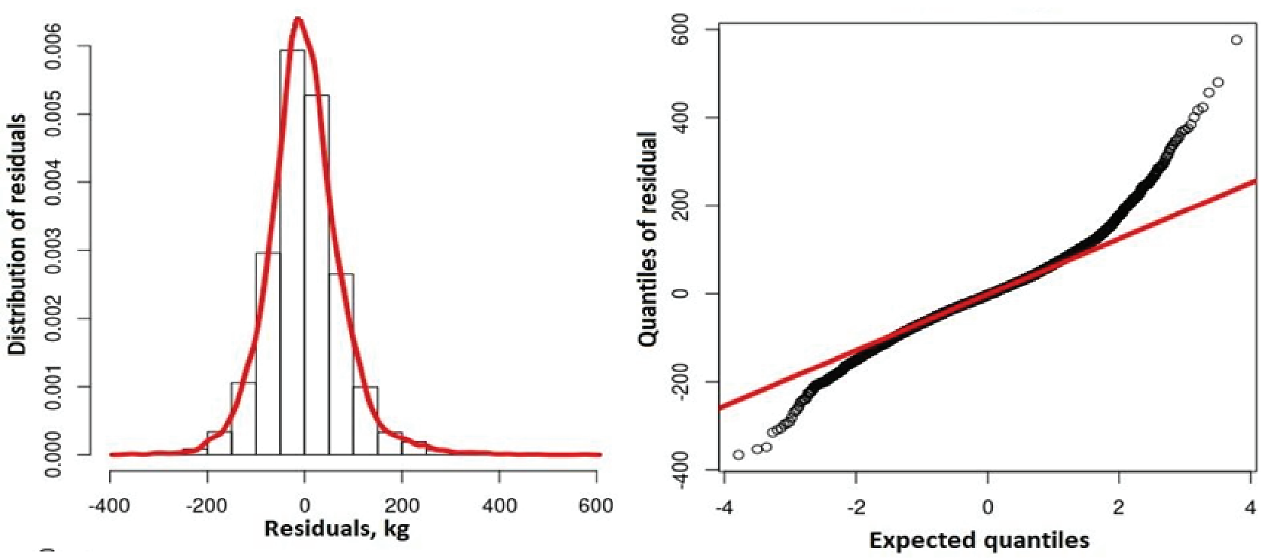


FIGURE 2. Correspondence between predicted and actual values for fat yield (%) and milk fat (kg)

A histogram of distribution and quantile-quantile plot was constructed to characterize the distribution of the residuals (Figure 3).



a) b)

FIGURE 3. Distribution of residuals: a) histogram; b) quantile-quantile diagram

The authors noted earlier in this paper, the difference in the values of the correlation coefficients led to the assumption of the presence of the residuals' outliers. This presence is confirmed by the fact of "tails" in the quantile distribution. However, the vast majority of observations were well described by the Gaussian distribution. Outliers were due to the sample data after truncation, which explained that a small proportion of the animals had lower than expected milk fat. However, this did not fully reflect their genetic potential but was a consequence of the adverse

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effects of animal feeding and keeping conditions. The authors believe that the predicted values more accurately reflect the breeding value of most such individuals, for which the residues showed boundary values.

Discussion

Low accuracy in predicting fat as a percentage may be due to the dependence of its calculation on milk yield. Usually, the actual milk yield and fat content are considered when determining the amount of milk fat. Fat content is subordinate to milk yield per 305 days of lactation. It is known that the weaker the selection, the higher the share of genotypic variation in a trait. The higher the share of variability, the more complex the feature is to predict [22]. Milk fat percentage (%) and milk fat in kilograms (kg) are quantitative traits. And in promising models, the interaction of genes that control these quantitative traits must be considered. In turn, gene expression is regulated by environmental conditions. The mixed regression model constructed included paratypic factors that significantly affect the variability of both traits. Some random effects that affect the gradations of the fixed elements were taken into account for the complex nature of the variability in milk fat content. Model values can more accurately describe breeding values than observed values in some cases. Thus, the authors consider it appropriate to give approximated model results in adult equivalent instead of empirical data in.

Conclusions

Studies have shown that fixed factors affect the variability of fat expressed as a percentage and in kilograms. Using Fisher's criterion, the predictors investigated can be ranked as follows: ELS (6661.2) – ELY (592.3) – CS: ELS (531.2) – CS (277.7) – CS (277.7) – CY (138.3) – CY: ELY (36.1) – CY: CS (11.2) – ELY: ELS (6.3) for the amount of milk fat (kg); CY (328.9) – ELY (42.8) – CY: CS (31.4) – CS: ELS (27.7) – CS (13.5) – ELS (8.4) – ELY: ELS (7.4) – CY: ELY (6.4). The prediction of milk fat in kilograms was more accurate than one in percent using the proposed model. The results suggest that traditional linear regression models to predict milk fat content are not always appropriate.

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