GLOBAL RISKS OF BIOLOGICAL INVASIONS OF PHYTOPATHOGENIC ORGANISMS AND IMPROVEMENT OF THE QUARANTINE MONITORING SYSTEM USING COMPUTER MODELING

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Abstract

As a result of assessing the distribution of adventitious species of phytopathogens on the territory of Belarus, a trend of increasing the number of recorded invasions was identified. Every year, an average of 3-4 new pathogens of infectious diseases of woody plants are identified in forests and gardens, and over the past 25 years, at least 57 new types of phytopathogens have been recorded. Many of the newly identified species have already passed the stage of acclimatization, and some have a significant impact on the sanitary condition of forests and parks, causing significant damage. With the Maxent software package, using the example of the phytopathogenic oomycete Phytophthora alni Brasier et S.A. Kirk, which is quarantined for the EAEU countries, a map of the current and future geographic distribution under various climate change scenarios was constructed. The models showed the suitability of the conditions of Belarus for the development of P. alni, which made it possible to conduct local forest pathological surveys and identify symptoms of alder late blight in various forest conditions of the country. Molecular genetic analysis confirmed the presence of a quarantine pathogen on the territory of Belarus. Forecasting the spread of the invader, on the one hand, shows a reduction in areas potentially suitable for the development of the species in Europe, and on the other hand, an increase in the likelihood of the invader moving to more northern areas. Considering the potentially high harmfulness of the species, immediate monitoring, analysis of phytosanitary risks and adoption of quarantine measures are already required.

Keywords: computer modeling, quarantine organism, Maxent.

I. Introduction

One of the most important reasons for modern global and local changes in the Earth's ecosystems is the accelerating development of human society. An increase in the planet's population and improving people's quality of life, on the one hand, lead to an increased use of natural resources, and on the other, due to such manifestations as the globalization of trade and communications, increased mobility of people, etc., creates the preconditions for large-scale violations of the «geobiotic order» [1, 2]. The most negative disturbances include invasions of harmful organisms (insects, fungi, bacteria, viruses, nematodes, vascular plants, etc.), their hybridization with local closely related species, displacement of native species, horizontal gene transfer, emergence of new races and species of pathogens, etc. etc. [3, 4]. Examples of global invasions are widely known in medicine and agriculture; they are no less destructive for native ecosystems, including forests. In relation to forestry and gardening, the result of invasions is the epiphytotic development of new diseases in natural and artificial phytocenoses, causing significant environmental and socio-economic damage [5].

Considering the enormous potential harmfulness that alien pathogenic organisms carry in new territories and in ecosystems that are different to them, scientists and practitioners in various fields of biology, agriculture, forestry, and medicine would like to have an idea about the likelihood of penetration and development of specific invaders in certain countries or regions. At the same time, quarantine measures based on national or regional lists of quarantine organisms are not always effective, among other things, due to the growing dynamics of the introduction of alien species [7] and some inertia of government bodies in updating these important documents [8].

Climate plays a fundamental role in the formation and changes in the habitats of living organisms [9]. Over the past few decades, the amount of information about climate change on a planetary scale has rapidly increased. They pose significant risks for various sectors of the economy and natural ecosystems [10]. Detailed and reliable information on the geographical distribution of species during global climate change is of great importance for various environmental protection, quarantine, protective and other measures [11]. To predict the potential for the settlement of alien species in new territories in order to assess phytosanitary risks, macro- and microecological studies are carried out taking into account the vectors of possible transfer of invaders [12].

One of the most popular tools for modeling the distribution of species in various environmental conditions is Maxent, a highly customizable software tool based on the principle of maximum entropy [13]. In the Google Scholar database the number of scientific articles published annually using Maxent to predict the potential ranges of species under climate change has nearly tripled over the past 10 years. Most studies show that compared with other niche models, the Maxent model has good predictive effect and stability [14]. The program's algorithms allow you to build a prediction model based on actual distribution points and environmental variables of the distribution area stored in the GIS and model the potential distribution of species in space. The result is a thematic map showing the suitability of areas for species distribution. In addition, Maxent helps to adapt environmental variables and evaluate the contribution of each variable [15, 16].

The purpose of this work was to audit the quarantine neobiota on the territory of Belarus and assess the predictive value of computer modeling for monitoring dangerous quarantine organisms in forests under various climate change scenarios.

II. Materials (objects) and research methods

Information about findings of quarantine phytopathogenic objects on woody plant species was tracked in open sources: scientific articles, international databases (GBIF, EPPO), reports and messages from government authorities on plant quarantine.

The phytopathogenic oomycete *Phytophthora alni* Brasier et S.A. Kirk. was chosen as a model object for spatial modeling of the neohabitat, included in the Unified List of Quarantine Objects of the Eurasian Economic Union as a pest not present on the territory of the Eurasian Economic Union.

A model of the potential spread of the quarantine pest *P. alni* and an assessment of the likelihood of its spread on the territory of the Republic of Belarus was built on the basis of data on the modern range of the invader obtained from authoritative international databases: the Global Biodiversity Information System (https://www.gbif.org/ru/occurrence/search) and the Euro-Mediterranean Plant Protection Organization (https://gd.eppo.int/taxon/PHYTRA/distribution).

Data on the relief and vegetation cover of the region, climatic factors were used for the analysis (http://due.esrin.esa.int/page_globcover.php). Raster files for 19 bioclimatic variables and altitude at 2.5 min resolution were downloaded from the Global Climate Database (http://worldclim.org/).

The predictive value of the model was tested through a selective forest pathological examination of alder plantations in various geobotanical conditions of the country on the territory of the Braslav Lakes National Park, Negorelsky educational and experimental forestry enterprise, Osipovichi experimental and Stolin forestry enterprises. Diagnosis of alder late blight was carried out using a set of symptoms and signs described on alder only with the development of *P. alni* [17].

Molecular genetic identification of the pathogen was carried out on 13 wood samples selected from symptomatic areas of alder trunks using the following methods: DNA extraction, classical PCR, Sanger sequencing, processing of nucleotide sequences in the BioEdit program and the NCBI database.

To extract DNA from wood, the DNEasy Plant Mini Kit (Qiagen) was used. Classical PCR was performed with isolated DNA samples. For DNA amplification, primers PA-F/PA-R were used, which amplify a product from 450 bp. The mixture of reagents for performing one reaction with a volume of 25 μl contained: 16 μl of RNA and DNA-free water, 5 μl of 5X PCR buffer MasDDTaqMIX – 2025 (Dialat Ltd., Moscow), 1 μl of each primer (10 μM) and 2 μl of target DNA. Temperature-time amplification parameters for primers ITS 4/ITS 5 included: predenaturation 95°C – 3 min, then 40 cycles consisting of denaturation 95°C – 30 sec, primer annealing 58°C – 30 sec, elongation 72°C – 60 sec; final pre-synthesis 72°C – 10 min.; storage at +4°C. The amplification results were recorded after electrophoresis in a 1,5% agarose gel stained with ethidium bromide in the Gel Doc XR+ gel documentation system (Bio-Rad). PCR product size was measured using GeneRuler™ 100+ bp molecular weight markers and Fast Ruler™ (Fermentas). PCR products intended for sequencing were purified using the commercial QIAquik PCR Purification Kit (Qiagen). The sequencing reaction was performed using BigDye Terminator v3.1 Cycle Sequencing Kit reagents (Applied Biosystems) according to the manufacturer's instructions, followed by fragment separation on an Applied Biosystems 3500 Genetic Analyzer.

III. Results and discussion

As a result of assessing the distribution of adventitious species of phytopathogens on the territory of Belarus, a trend towards an increase in the number of recorded invasions at the turn of the 20th and 21st centuries was identified (Fig. 1). Currently, an average of 3-4 new pathogens of infectious diseases of woody plants are identified annually in forests and gardens, and at least 57 new species of phytopathogens have been recorded over the past 25 years. Many of the newly identified species have already passed the stage of acclimatization, and some have a significant impact on the sanitary condition of forests and parks. For example, the appearance on the territory of the country of the East Asian pathogen *Hymenoscyphus fraxineus* (T. Kowalski) Baral, Queloz & Hosoya) is associated with the death of more than ¾ of the country’s ash forests [18] and enormous damage to forestry [19].

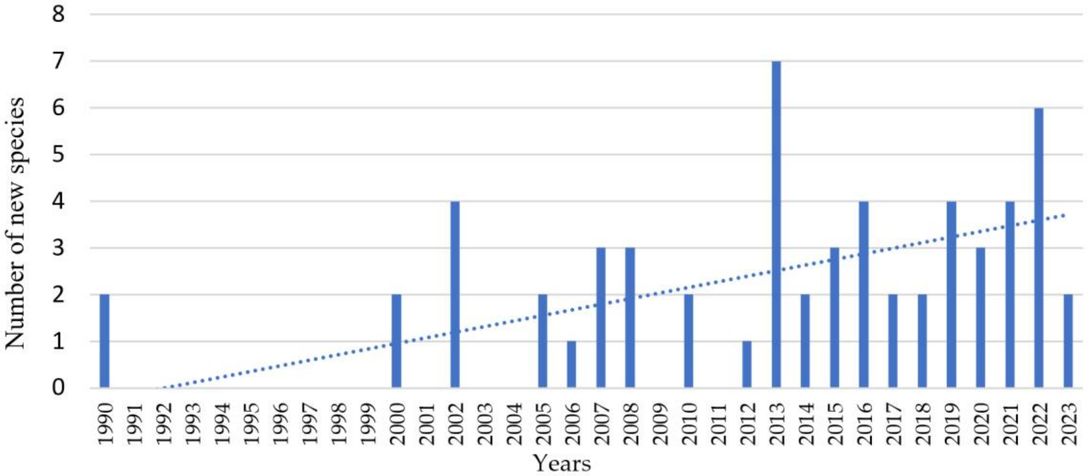

Fig. 1: *Dynamics of detection of new invasive species on the territory of Belarus*

The Unified List of Quarantine Objects of the Eurasian Economic Union (EAEU), which applies to Armenia, Belarus, Kazakhstan, Kyrgyzstan and Russia, includes 19 types of phytopathogens dangerous to woody plants. Moreover, 16 of them are marked as absent from the territory of the EAEU as of March 2023. However, a review of the literature shows that this is not accurate data, and at least 4 pathogens from this list are more or less common in the territories of the union countries (Table. 1). In addition, the list has some inaccuracies in the taxonomic nomenclature of species (legitimate names are indicated in brackets), which indicates a lack of close attention to this document on the part of the supervising government bodies of the EAEU countries.

Table 1: *List of quarantine phytopathogens of woody plants according to the EAEU and information on their identification on the territory of the Eurasian Economic Union*

№	Name of the pest quarantine organism from the EAEU (modern name)	Disease caused	Host plants	Information about identification in the EAEU	Conclusion
1	*Atropellis pinicola* Zeller & Goodding	branch canker of pine	*Pinus sylvestris,* *P. monticola,* *P. lambertiana,* *P. contorta, P. strobus*	No information	Not identified
2	*Atropellis piniphila* (Weir.) Lohman & Cash	branch and trunk canker of pine	*Pinus albicaulis,* *P. banksiana,* *P. contorta, P. ponderosa*	No information	Not identified
3	*Ceratocystis fagacearum* (Bretz.) Hunt	oak wilt	*Quercus spp.*	No information	Not identified
4	*Chalara fraxinea* T.Kowalski (*Hymenoscyphus fraxineus* (T. Kowalski) Baral, Queloz & Hosoya)	ash dieback	*Fraxinus spp.*	Belarus: Zviagintsev, Sazonov, 2006 [20]; Zviagintsev et al., 2011 [21]; Russia: Musolin et al., 2014 [22]; Zviagintsev et al., 2015 [23]; Zviagintsev et al., 2023 [24].	Belarus is a ubiquitous species; Russia – a ubiquitous species in its natural range (Far East) and as an invader throughout the European part of Russia with the presence of host breeds

5	*Cronartium fusiforme* Hed. & Hunt ex Cum.	fusiform rust of pine	*Pinus spp., Quercus spp.*	No information	Not identified
6	*Cronartium quercuum* (Berkeley) Miyabe ex Shirai	pine-oak gall rust	*Pinus spp., Quercus spp., Castanea spp.*	Russia: Kaneko, 2000 [25].	Considered a native species in the Russian Far East
7	*Gymnosporangium yamadae* Miyabe ex Yamada	Japanese rust of apple	*Malus spp., Abies spp., Juniperus spp.*	No information	Not identified
8	*Melampsora medusae* Thümen	leaf rust of poplar	*Larix spp., Picea spp., Pinus spp., Pseudotsuga menziesii, Tsuga spp., Populus spp.*	No information	Not identified
9	*Mycosphaerella dearnessii* ME Bar (*Lecanosticta acicola* (Thüm.) Syd.)	brown spot of pine	*Pinus spp.*	Belarus: Golovchenko et al., 2020 [26] Russia: Violin, Surina, 2013 [27]	Belarus - common in arboretums on decorative pine species; Russia – Black Sea coast and Sakhalin.
10	*Mycosphaerella gibsonii* HC Evans (*Pseudocercospora pini-densiflorae* (Hori & Nambu) Deighton)	brown needle blight of pine	*Pinus spp.*	No information	Not identified
11	*Mycosphaerella laricis-leptolepidis* K. Ito, K. Sato & M. Ota	needle cast of Japanese larch	*Larix spp.*	No information	Not identified
12	*Phytophthora alni* Brasier & SA Kirk	root disease of alder	*Alnus cordata, A. glutinosa, A. incana, A. viridis*	A single find in the southeast of Belarus in 2014 [28]	Single outbreak
13	*Phytophthora kernoviae* Brasier	late blight of ornamental and tree crops	*Fagus sylvatica, Rhododendron ponticum, Drimys spp., Magnolia spp., Michelia spp., Quercus spp., Vaccinium spp.*	No information	Not identified
14	*Phytophthora ramorum* Weres et al.	ramorum leaf blight	*Fagus sylvatica, Quercus spp., Larix kaempferi. L. decidua*	No information	Not identified
15	*Sirococcus clavigignenti-juglandacearum* Nair, Kostichka & Kunt (*Ophiognomonia clavigignenti-juglandacearum* NBNair, Kostichka & Kuntz)	canker of butternut	*Juglans ailanthifolia, J. ailanthifolia var. cordiformis, J. cinerea, J. nigra, J. regia*	No information	Not identified
16	*Bursaphelenchus xylophilus* (Steiner & Buhrer) Nickle	pine wilt disease	*Abies spp., Cedrus spp., Larix spp., Picea spp., Pseudotsuga spp.*	No information	Not identified

Some quarantine species from this list, for example *Atropellis pinicola* and *A. piniphila*, are absent from Europe, others are recorded only in certain countries (*Bursaphelenchus xylophilus*, *Melampsora medusae*) but some were able to spread quickly, creating huge secondary habitats. An example of the latter is the causative agent of alder late blight the oomycete *P. alni*. In Belarus, the first single plant with symptoms of the disease was identified back in 2014 on the territory of the Gomel forestry enterprise [28]. The presence of a quarantine pathogen was confirmed using molecular genetic diagnostic methods, but since then the symptoms of the disease have no longer been diagnosed in Belarus. A working hypothesis has been put forward that the environmental conditions of the country turned out to be unfavorable for the development of *P. alni*, and the phytopathogen could not go through the acclimatization stage and spread from the site of the supposed single introduction. To test the hypothesis using computer modeling, a *P. alni* distribution model was adjusted by correlation analysis of 19 bioclimatic variables, which was performed using QGIS and Excel to eliminate multivariate collinearity. Bioclimatic variables that showed a coefficient of contribution to the species distribution greater than 0 in Maxent calculations were selected for analysis. The most significant environmental variables were used for further modeling (Table 2).

Table 2: *Environmental variables shown to be most important for the distribution of Phytophthora alni in Maxent calculations*

Variable	Description
BIO7	annual temperature range
BIO8	average temperature of the wettest quarter
BIO10	average temperature of the warmest quarter
BIO14	precipitation of the driest month a
BIO15	seasonality of precipitation (coefficient of variation)
BIO16	precipitation of the wettest quarter
BIO18	precipitation of the warmest quarter
elevel	terrain data
cover	land cover data

To assess the potential distribution of the study species under the influence of climate change, a model developed jointly with the Japanese research community, known as the Interdisciplinary Research Climate Model (MIROC 6), was used with a resolution of 2.5 minutes or ~5 km per pixel, which is often used to solve wide range of issues in climate science and future climate projections. The MIROC 6 forecasting system is a contribution to phase 6 of the CMIP 6 project (Coupled Model Intercomparison Project, Version 6).

The modeling used two extreme climate scenarios according to the 6th report of the Intergovernmental Panel on Climate Change: SSP 585 – an option providing for the highest concentration of carbon dioxide in the future, SSP 126 – an option providing for the lowest concentration of carbon dioxide in the future [29].

When carrying out the analysis the following settings were set in the Maxent program: for statistical analysis of accuracy, the resulting models were tested by a random sample of 25% of species locations, 500 steps (iterations) were used to obtain the optimal model, the complexity parameter (regularization multiplier) equal to 1 was selected experimentally based on the analysis of different models, the maximum number of background points – 15000, random subsamples were selected based on cross-validation.

The most important tool for checking the reliability of the constructed model is the AUC value (Area Under the Receiver Operating Characteristic – area under the receiver operating characteristic). The AUC of the ideal model is 1, but adjusted for «presence only data», the AUC indicator of a good model tends to 1 [30].

The criteria for assessing the modeling accuracy of the model were divided into three levels: poor (AUC ≤ 0,50), acceptable (0,5 < AUC ≤ 0,80) and excellent (0,80 < AUC ≤ 1,00) [31].

In our case, the values Training data AUC = 0,921, Test data AUC = 0,784, for model *P. alni* indicate a fairly high level of predictive efficiency.

The importance of the variable was assessed using the jackknife test, which took into account the environmental factor most influential on the spread of *P. alni* (Fig. 3).

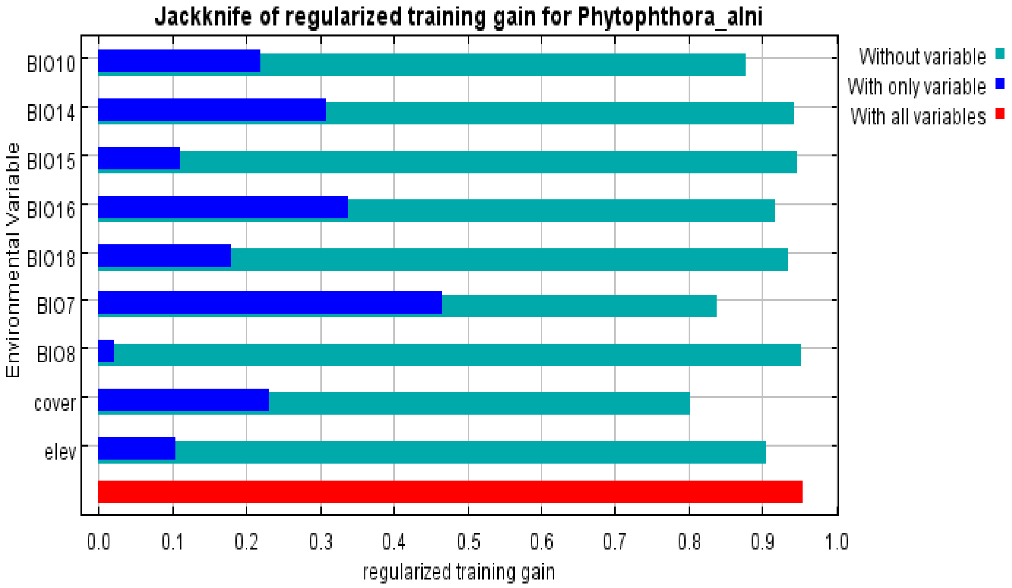

Fig. 2: *Results of the jackknife test*

The results show that predictors such as BIO 7 (annual temperature range), cover (vegetation zones), BIO 14 (precipitation of the driest month) and BIO 16 (precipitation of the wettest quarter) contributed the most to the distribution pattern of *P. alni* compared to other variables.

Temperature conditions are a more important parameter than altitude. This suggests that climate in Europe is a powerful driver of the spread of *P. alni*. A variable associated with extreme environmental conditions (precipitation from the driest month) has also been found to be important in explaining the spread of this plant pathogen, as rainfall affects the amount of water in rivers, streams and marshes, which is closely related to the distribution of the alder host plant.

The result of the modeling is maps of the possible distribution of *P. alni* at the current moment and in the future, colored according to the optimal environmental conditions for the development of the species (Fig. 4). In the scale used, red corresponds to the most favorable conditions, and blue to unfavorable conditions, where the probability of development of a given species tends to 0.

Spatial and climatic modeling of the *P. alni* range shows the suitability of the conditions of Belarus for the development of the pest with probability coefficients from 0,22 to 0,78. Under changing environmental conditions under different climate scenarios, the species in question will migrate towards higher altitudes and latitudes.

All SDM forecasts were visualized in QGIS. To more clearly assess future changes in niche area compared to the current model, presence/absence maps were constructed using the minimum presence threshold during training (0,47). Suitable habitats were ranked, and their area was calculated (Figure 5). Further, with area measurements, they were divided into three classes: the most suitable habitat (0,65 < P ≤ 1,0), highly suitable habitat (0,47 < P ≤ 0,65) and unsuitable habitat (P ≤ 0,47) (Table 3).

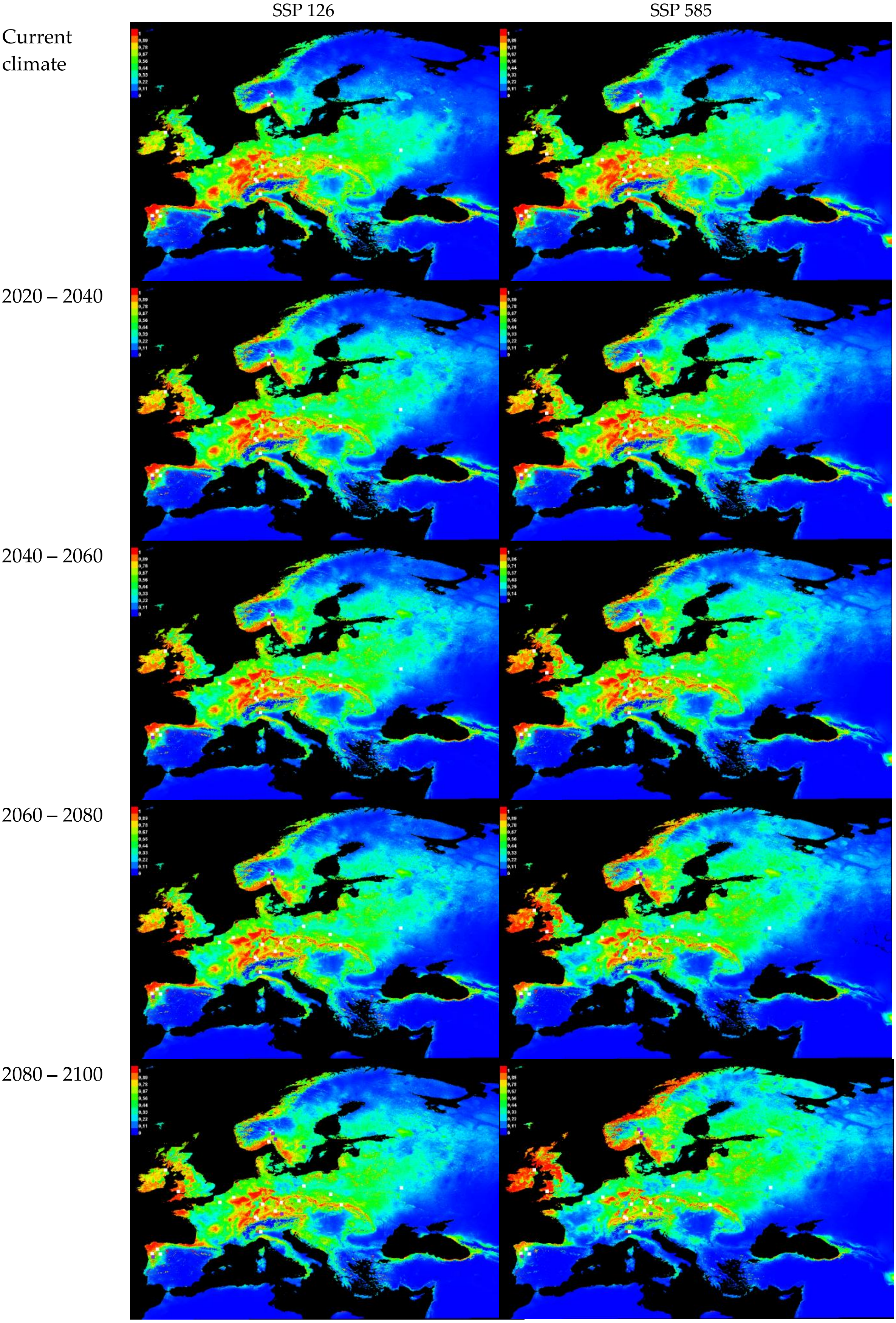

Fig. 3: *Temporal forecasting of changes in the favorable environmental conditions for the development of the potential range of Phytophthora alni*

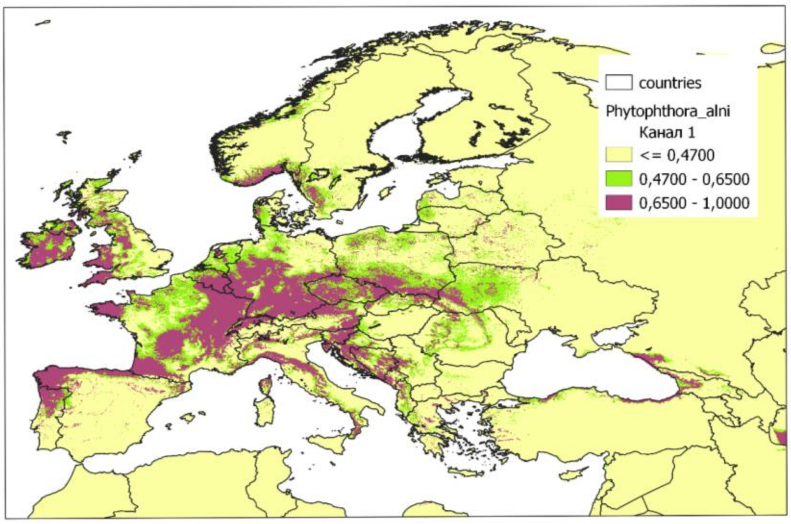

Fig. 4: *An example of territory classification according to suitability categories*

Table 3: *Area of predicted habitats depending on various climate change scenarios, thousand km*^{2}

Probable habitats	Current climate	Forecasting depth, years							
		SSP 126				SSP 585			
		20-40	40-60	60-80	80-100	20-40	40-60	60-80	80-100
most suitable	1,280	1,289	1,199	1,138	1,083	1,354	1,237	1,057	0,965
highly suitable	1,059	0,999	0,961	0,927	0,938	1,049	2,027	0,914	0,866
unsuitable	9,626	9,545	9,664	9,784	9,803	9,421	9,591	9,846	10,407

The numbers presented above once again prove that in general for Europe the territory is suitable for naturalization and potential spread invader will be reduced. This can be associated with the continuing increase in climate aridity and desertification of the southern regions of the subcontinent [32]. At the same time, on the territory of Belarus, under «optimistic» and «pessimistic» climate change scenarios, the habitat will change towards greater suitability for *P. alni.*

P. alni grow naturally in plantations in Belarus: *Alnus glutinosa* and *A. incana*, whose plantings occupy about 9% and 2% of the forested area, respectively [33]. The area of alder forests is more than 900 thousand hectares with a timber reserve of 180 million m^3. Alder forests occupy mainly low-lying relief elements, often along ponds and streams, where the probability of the spread of the oomycete *P. alni* is high. Selective forest pathological examinations of alder plantations made it possible to identify symptoms of late blight in the protected zone of the Braslav Lakes National Park, Osipovichi end Stolin forestry. In most outbreaks, the disease manifested itself on individual weakened or drying trees. In localities in the south and central part of the republic, the occurrence of symptoms can be characterized as single. Relatively massive damage to alder was detected in the plantations of the Braslav Lakes National Park, where symptoms of late blight were recorded in 11 forest areas with dominance or participation in the composition of *A. glutinosa* and *A. incana*, and in some cases group damage and death of trees were noted. The discovery points were located at different distances from reservoirs and watercourses, which casts doubt on the exclusively hydrochoric mode of spread of the pathogen.

Affected alder plants experience defoliation, the leaves become small and fall off prematurely. Dryness is often noted. Black or dark red wet spots form in the lower part of the trunks (up to a height of 2–3 meters). Dark exudate is periodically observed on the surface of the necrotic bark (Fig. 5).

Fig. 5: *Symptoms of black alder late blight: on the bark - on the left; under the bark - on the right (Osipovichi forestry, 2022)*

The spots indicate the death of the cambium and wood under the bark as a result of the development of a pathological process. When the phloem is removed, elongated zones of brownish-brown color are visible, which contrast with healthy wood, which is white when freshly cut. Over time, the affected bark and wood are destroyed.

Molecular genetic analysis confirmed the presence *of P. alni* in Belarus. And amplification with primers PA-F/PA-R made it possible to obtain a target fragment of 450 bp in size with a sample from the Osipovichi forestry (central part of Belarus). These primers are specific for all forms of *Phytophthora alni* (*P. alni subsp. alni, P. alni subsp. uniformis and P. alni subsp. multiformis*).

Also, when performing PCR, nonspecific products were obtained with three samples. In this regard, further identification of samples was carried out using Sanger sequencing. As a result of analysis of the obtained nucleotide sequences in the NCBI database, it was found that the sample from the Osipovichi forestry is *Phytophthora alni subsp. alni*. Percent identity with *Phytophthora alni subsp. alni* sequence isolate UASWS 0194 SCAR marker F 4-1050 genomic sequence (Sequence ID: DQ 403799.1) is 99,10%.

High-quality alder timber is in high demand in the domestic and foreign markets; thanks to its rich texture, it is a source of high-quality raw materials for the production of plywood, furniture, doors, and interior elements [34]. Considering the scenarios for the development of pathology in European countries, one can expect a significant volume of alder plantations damaged and weakened by the disease. Due to the inaccessibility of such areas during most of the year, prompt implementation of sanitary and health measures in these conditions will be difficult. According to an assessment based on the regulations of the International Standard for Phytosanitary Measures [27], when mass drying of alder plantations occurs, the potential damage to forestry is assessed as significant. When alder forests are damaged and dry out, we should expect waterlogging and deforestation of areas in which alder is the main forest-forming edificator.

IV. Discussion

The global trend of increasing the number of invasions of all taxonomic groups of living organisms into new regions is especially clearly manifested in native and artificially created ecosystems of Belarus using the example of dendropathogens. Currently, an average of 3-4 new pathogens of infectious diseases of woody plants are identified annually in forests and gardens, and at least 57 new species of phytopathogens have been recorded over the past 25 years. Many of the newly identified species have already passed the stage of acclimatization, and some have a significant impact on the sanitary condition of forests and parks, causing significant environmental and socio-economic damage. This indicates the low effectiveness of the current system of measures to curb biological invasions at the regional and global levels.

Computer modeling is a convenient and promising tool for solving the problems of predicting the development of secondary habitats of quarantine pests in existing environmental conditions, both currently and under various climate change scenarios. The constructed forecasts can be used for high-quality analysis of phytosanitary risks, development of a set of effective quarantine measures and organization of operational monitoring of outbreaks of quarantine objects in the country's forest fund.

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