

ASSESSMENT OF THE SPECIFIC DISEASES IN *Reynoutria sachalinensis* (F. Schmidt) Nakai UNDER THE INFLUENCE OF ENVIRONMENTAL CONDITIONS OF THE REPUBLIC OF MOLDOVA

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Abstract

A major risk for the species *Reynoutria sachalinensis*, as a non-traditional honey, fodder and bioenergy crop, in the Republic of Moldova, are the invasive diseases that affect the plants in the first growing season, once the assimilation organs are formed. It is an early-emerging species, subjected to the influence of stress factors, including diseases that affect the growth potential of plants. The research carried out in 2015-2022 by complex phytosanitary monitoring highlighted the spread of some pathogens that affect the plants of *R. sachalinensis* annually or periodically, being also favoured by environmental factors. We assessed the most significant diseases detected throughout the growing season, caused by pathogens such as: *Peronospora fagopyri*, *Erysiphe communis* f. *polygoni*, *Septoria ascophylli*, *Ascochyta fagopyri*, *Phyllosticta polygonorum*, *Botrytis cinerea*, *Sclerotinia sclerotiorum*, *Fusarium gibbosus*, *F. oxysporum* f. *sp. spinacia*, *Cucumis virus*, *Tabacco mosaic virus* (TMV). A total of 11 diseases were recorded, 9 of which were of fungal origin and 2 of viral origin, causing various pathologies of etiological nature, of various frequency and significant intensity of damage caused to *R. sachalinensis* plants.

Key words: *Reynoutria sachalinensis*, pathogens, phytosanitary monitoring, pathogens, diseases.

INTRODUCTION

The major significance of the non-traditional fodder crop, introduced in the Republic of Moldova, *Reynoutria sachalinensis* (F. Schmidt) Nakai, common names - giant knotweed or Sakhalin knotweed, lies in its multiple uses and advantages for the local flora, being attributed to the plants with high estimated potential as honey, energy and ornamental crops (Teleuță et al., 2013; Țîței & Teleuță, 2014).

This species, taxonomically, falls in the order Polygonales, family Polygonaceae Juss., which comprises over 1000 species of herbaceous, annual and perennial plants, which are present worldwide, with some exceptions such as New Zealand and Antarctica (Hrjanovski & Ponomarenko, 1993). *R. sachalinensis* is native to East Asia - northern Japan, China, Korea, including the far east of the Russian Federation, and was later introduced to Western Europe (1855) (Nakai, 1926; Mandak et al., 2004).

Later it spread to large areas of the American continent, particularly in USA, where it was grown to explore its bioecological and forage potential, and for the first time it was described back in 1859, in the book “Primitise Floral Amurensis” (Bailey & Conolly, 2012), and then, successively, over time, it was described in various specialized bibliographic sources by many botanists, focusing on its biomorphological and ecological characteristics, as a species used for various purposes: fodder, honey, medicinal, energy, food, ornamental, ecological (Vavilov & Condratiev, 1975; Schnitzler & Muller, 1998; Kawai et al., 2004; Țîței & Teleuță, 2014; Ivanov, 2015; Calalb et al., 2017). The possibilities of using this species as source of fodder and bioenergy are of particular importance for the agro-industrial branch of the Republic of Moldova. The above-mentioned advantages were important reasons of introducing this species in the Republic of Moldova (in 1982); the initial material

(rhizomes) was brought by Teleuță A., PhD, from the Agricultural Institute of Vladikavkaz (The Republic of North Ossetia - Alania, Russian Federation).

In terms of morphological aspects, it is a perennial, herbaceous plant, with erect stems, hollow at the internodes and simple, petiolate leaves, with broad-ovate blade, 20-38 cm long and 15-20 cm wide, with acuminate tip and slightly wavy margin. The leaves have dense hairs on the lower epidermis and are alternately positioned on the stem. The flowers are small, actinomorphic, with simple perianth, produced in panicle inflorescences. The fruits are 3-sided achenes, 2.8-4.5 mm long and 1.1-1.8 mm wide (Fuentes et al., 2011; Cîrlig, 2019; Țiței & Roșca, 2021).

The fast progress of science and technology imposes the objectives of continuously obtaining agricultural productions by means of all plant species with high estimated potential as energy and feed crops, for domestic and wild animals; however, the favourable environmental conditions are advantageous not only to plants but also to various complexes of harmful organisms that act as a limiting factor in increasing the productivity of plants, that is why, as part of an extensive study on plants, some research on biological and phytosanitary control, including the diseases associated with the application of some remedies to regulate the pathological conditions in plants during the cultivation process, is also needed (Bobeș et al., 1972; Baicu & Șăvescu, 1986; Docea & Severin, 1990; Popescu, 2005; Volosciuc, 2009;). The harmful impact of diseases on the productivity of agricultural crops can be enormous, being also facilitated by the current unstable environmental conditions, because of which, science is currently progressing towards the creation of new plant cultivars, characterized by resistance and tolerance to the action of pathogens that triggers invasive diseases with considerable consequences for the agriculture, animal husbandry, forestry and socioeconomic sector (Semencova & Socolova, 1992, Bădăraș & Bivol, 2007; 2013). This species, being a physiologically precocious plant, with long growing season, abundant vegetative and generative growth, with fast regeneration of the green mass, has multiple indisputable qualities, but there are also significant manifestations of

the consequences caused by phytopathogenic agents, which cause specific diseases, including those acquired through specializations and adaptations of other pathogenic infections, detected practically throughout the growing season, with adaptations facilitated by the favourable environmental conditions of the Republic of Moldova, a fact that motivated us to carry out complex research on the diagnosis of diseases in this species, their etiological features, establishing the frequency and severity of these diseases in *R. sachalinensis* plants. Because of the topicality of the addressed issues, the goal of our research was: the phytopathogenic monitoring of the species *R. sachalinensis*, in correlation with the pedoclimatic conditions of the Republic of Moldova. We set the following *objectives*: keeping biological and phytosanitary control records of the establishment of pathogen complexes and the diseases triggered in the *R. sachalinensis* species, grown under experimental conditions at the "Alexandru Ciubotaru" National Botanical Garden (Institute); establishing the comparative values characterizing the indices of frequency, intensity and severity of damage caused by the phytopathological impact of the detected diseases in this plant species.

MATERIALS AND METHODS

The cultivar 'Gigant' of Sakhalin knotweed, *Reynoutria sachalinensis* (F. Schmidt) Nakai (Figure 1), patented and included in the Register of Plant Varieties of the Republic of Moldova in 2012, served as research subject.

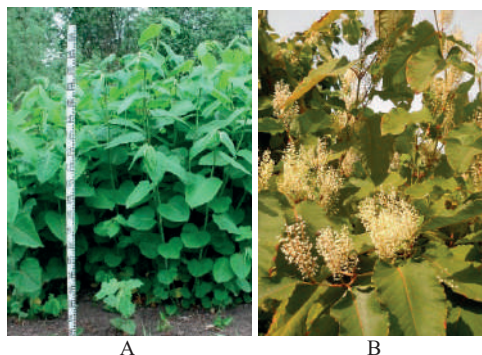


Figure 1. *R. sachalinensis* in the collection of "Alexandru Ciubotaru" National Botanical Garden (Institute):
A - vegetative phase; B - generative phase

Complex phytopathological monitoring was carried out at the "Alexandru Ciubotaru" National Botanical Garden (Institute), in the experimental sector of the "Plant Resources" Laboratory, on an area of 0.25 ha, planted with *R. sachalinensis* aged 10 years.

The planting scheme on the experimental sector was: 70 x 70 cm and 70 x 100 cm distance between rows and between plants, on a flat land area located between poplar shelterbelts and the collection of *Junglans* sp. The phenological observations were carried out through periodic surveys, according to the requirements of the methodological guidelines for the detection of harmful organisms in cultivated plants and elements of integrated protection in the Republic of Moldova, 2002 (Bădăraș, 2009).

The research activity was carried out by comparing the annual evolution, in 2015-2022. The essential role in the evolution of pathogenic infections affecting *R. sachalinensis* plants was played by the environmental factors characterized annually by high amounts of precipitation, more frequently recorded in April-May, and moderate air temperatures. These conditions were favourable for the fast growth and development of *R. sachalinensis* plants, as well as some phytopathogenic agents, which attacked the plants from the end of May, in the stage of formation of young shoots and foliage until the seed ripening stage, in October.

We conducted special surveys on the evolution of diseases, by assessing the level of invasive impact. Samples were taken at equal distances, a total of 50 plants on the diagonal of the experimental sector (rectangular shape) for each monthly survey, focusing on newly formed shoots, leaves, inflorescences, stems, roots, especially those visibly affected, in the dynamics of the development phases, by carefully highlighting the etiological and symptomatic aspects - the macroscopic field method.

The affected organs were collected and later analysed in the laboratory, applying the humidity chamber method (Figure 2), for 48 hours, and then studied under a microscope, consulting the key identification guidelines of cultivated plants and spontaneous flora according to: Hohreacova et al., 1984; Docea & Severin, 1990; Bădăraș & Bivol, 2007;

Bădăraș, 2009. Later, by calculations, the parameters characterizing the resistance of plants to infectious pathogens were established, determining the degree of attack, using the percentage formula:

$$G_a = \frac{F\% \times I\%}{100}$$

where: *F%* - frequency, and *I%* - intensity.

Preventively, for the estimation of these values, disease frequency (*F%*) was calculated, representing the relative value of the number of affected plants or organs (*n*) per total number of analysed plants or organs (*N*).

$$F\% = \frac{n \times 100}{N}$$

Another significant parameter is disease intensity (*I%*), which indicates the intensity of the development of a disease as a percentage, that is, to what extent a plant or organ was affected. To calculate the qualitative index of the disease intensity, the following formula was used:

$$I\% = \frac{(n_1 \cdot 1) + (n_2 \cdot 2) + (n_3 \cdot 3) + (n_4 \cdot 4)}{N \times 4}$$

where: *n*₁, *n*₂, *n*₃, *n*₄ - the number of plants or organs affected at the respective grade; *N* - the total number of examined plants or organs; 5 - the maximum grade of the scale.

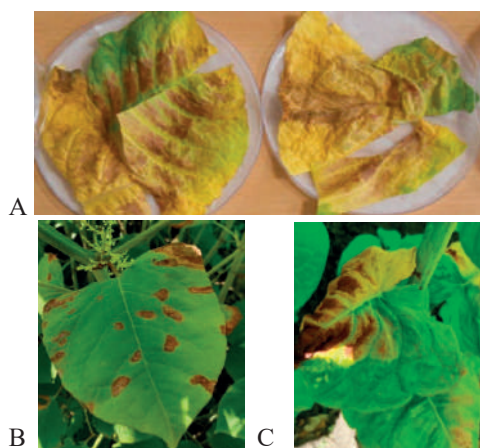


Figure 2. A - establishing the diagnosis on leaves with pathological conditions exposed in humidity chambers (Petri dishes); B - leaves affected by *Septoria ascophylli*; C - leaves affected by *Phyllosticta polygonorum*

In our experiments, the scale with 5 grades was used to denote the disease intensity, which corresponds to certain intervals that express the percentage of the affected area, namely: 0 - visible symptoms are absent; 1 - the affected

surface is up to 10%; 2 - the affected surface is from 10 to 25%; 3 - the affected surface is from 25 to 50%; 4 - more than 50% of the surface of the organ is affected. The obtained results were integrated in tables, diagrams and conclusive analyses.

RESULTS AND DISCUSSIONS

R. sachalinensis is an herbaceous perennial with hollow, erect stems at maturity, which, under the climatic conditions of the Republic of Moldova, reach a maximum height of 5-6 m. Underground organs are creeping rhizomes with thin adventitious roots. The flowers are small, cream-white, grouped in panicles. The fruits are 3-sided, brown achenes.

In the researched plants, during the growing season, the symptoms of the infectious process caused by pathogens were specific morpho-anatomical and physiological changes. For the most part, the specific symptoms that accumulated, became visible, and based on them, the specific external symptoms of the disease and the clinical picture of affected plants were established. Obviously, through the evolution of the vegetative stages under the influence of the environmental factors, certain changes in the aspect of the plants occurred and differentiated the forms of manifestation of the detected diseases. Based on the surveys and analyses, the symptoms of specific diseases, signalled by local staining of the tissues on different organs, wilting, chlorosis, browning, mosaic spots, covered with efflorescence (a fine white, grey, cream, pink down) on the upper epidermis and lower, young shoots, stems at internodes and nodes, it was possible to establish the diagnosis and preliminary pathogen, and later through laboratory analyses followed by microscopic analyses, the pathogen and its taxonomic affiliation were determined. But as some diseases such as downy mildew, powdery mildew, septoria, forms of white, grey and dry rot, through evolutionary changes, led to the death of the affected organs. And certain parameters characterizing the frequency and intensity of the disease, related to the degree of damage were reflected in Table 1. For the first time, 11 diseases with pathological impact were diagnosed in *R. sachalinensis* plants, under the

conditions of the Republic of Moldova, among which, two are of viral origin and 9 of fungal origin. The diseases detected during the active growth period, the stages of shoot formation - full ripening of seeds (May - October) and the estimation of the taxonomic affiliation of the pathogen by making records and pathological diagnosis are presented below.

Table 1. The etiological composition of the diseases and pathogens detected in the species *R. sachalinensis*

№	Name of the disease	Pathogen	Taxonomic affiliation
1	Downy mildew	<i>Peronospora fagopyri</i> Elenov (Berk) de By	Cl. Oomycetes Ord. Pythiales
2	Powdery mildew	<i>Erysiphe communis</i> (Wall) Grev. f. <i>polygoni</i>	Cl. Ascomycetes Ord. Erysiphales
3	Septoria leaf spot	<i>Septoria ascophylli</i> Melnic & M. Petrov	Cl. Deuteromycetes Ord. Capnodiales
4	Ascochyta blight	<i>Ascochyta fagopyri</i> Bres	Cl. Deuteromycetes Ord. Pleosporales
5	Phyllosticta leaf spot	<i>Phyllosticta polygonorum</i> Sacc.	Cl. Deuteromycetes Ord. Botryosphaeriales
6	Grey mould	<i>Botrytis cinerea</i> Fr., pv. <i>fagopyri</i>	Cl. Deuteromycetes Ord. Hyphales
7	White mould	<i>Sclerotinia sclerotiorum</i> (de By) Korf. Et Dumont.	Cl. Ascomycetes Ord. Helotiales
8	Stem rot	<i>Fusarium gibbosus</i>	Cl. Deuteromycetes Ord. Hyphales
9	Root and stem rot	<i>F. oxysporum</i> f. sp. <i>spinacia</i> W.C. Snyder & H.N. Hansen	Cl. Deuteromycetes Ord. Hyphales
10	Mosaic chlorosis	<i>Cucumis virus I</i> Smith	Cl. Eucristallinea Ord. Insectophiles
11	Tobacco mosaic disease	<i>Tobacco mosaic virus</i> (TMV)	Cl. Alsuviricetes Ord. Martellivirales

Simultaneously with the pathographic, pathogenic and epidemiological research on Sakhalin knotweed plants, the biological control regarding the qualitative and quantitative aspects of the invasive impact of viral and fungal agents, detected on various plant organs, was carried out.

The field analyses on the researched plants highlighted the manner of manifestation and extent of the diseases through certain specific symptoms, taking into account the individual contact with each phytopathogen, causing diseases of different severity, characterized by: discolouration, chlorosis, spots, browning,

followed by organ deformation, partial or total necrosis of the foliage and stems. These symptoms facilitated the determination of the diseases that affected this plant species, which, under the conditions of the Republic of Moldova, was contaminated by downy mildew, *Phyllosticta* leaf spot, *Ascochyta* blight, powdery mildew, grey mould, white mould, root and stem rot, mosaic chlorosis, reported more abundantly in ascending aspect on all plant organs.

These associations of pathogens drew the attention of researchers, who frequently conduct phytosanitary surveys, which are included in research programs aimed at introducing traditional and non-traditional fodder, honey and energy crops characterized by resistance and tolerance to environmental stress factors.

The research carried out was completed with records and analyzes related to parasitic impact indices characterizing the degree of individual

attack of each detected pathogen, with qualitative and quantitative impact indices.

Resulting from the instability of the climatic factors of the last decade, in the Republic of Moldova, there is an alternation of periods with high amounts of precipitation and diurnal temperatures, annually, between April and May, a fact that conditioned the spread of the following specific diseases that initially appear in the period of foliage formation, such as: downy mildew *Perenospora fagopyri* Elenov, with the highest frequency of 60 % on leaves, followed by powdery mildew *Erysiphe communis* (Wall) Grev. f. *polygoni*, which was detected later, in summer drought periods, with 50 % frequency, which associated in the middle of summer with *Septoria* leaf spot *Septoria ascophylli* Melnic & M. Petrov - 40%, *Ascochyta* blight *Ascochyta fagopyri* Bres - 30% and *Phyllosticta* leaf spot *Phyllosticta polygonorum* Sacc. - 24% (Table 2).

Table 2. The etiological composition and the level of invasive impact with the diseases detected in *R. sachalinensis* (2015-2022)

Disease	Frequency (%)	Intensity (%)	Degree of attack (%)	Affected organs
<i>Perenospora fagopyri</i>	60	0.28	0.17	leaves, young shoots
<i>Erysiphe communis</i> f. <i>polygoni</i>	50	0.27	0.14	leaves, young shoots
<i>Septoria ascophylli</i>	40	0.20	0.08	leaves
<i>Ascochyta fagopyri</i>	30	0.14	0.04	shoots
<i>Phyllosticta polygonorum</i>	24	0.11	0.03	leaves, shoots
<i>Botrytis cinerea</i> pv. <i>fagopyri</i>	18	0.10	0.02	the base of stems
<i>Sclerotinia sclerotiorum</i>	28	0.11	0.03	internodes, nodes
<i>Fusarium gibbosus</i>	16	0.08	0.01	Mature stems
<i>F. oxysporum</i> f. sp. <i>spinacia</i>	12	0.05	0.006	stems, old roots
<i>Cucumis virus</i>	12	0.06	0.007	young leaves
<i>Tobacco mosaic virus (TMV)</i>	10	0.05	0.005	mature leaves

All diseases have significant impact, causing severe damage to annual leaves and shoots in the first growing season. In the second growing season, under the influence of environmental factors, other diseases were also detected, such as: forms of grey and white mould, fusarium rot, favoured by the amount of precipitation and diurnal temperature alternations.

At the same time, two diseases of viral origin were detected: *Cucumis virus* I Smith and *Tobacco mosaic virus* (TMV), with and incidence of 12% and 10%, with symptoms such as mottling and distortion (curling) of mature leaves, being caused by vector insects such as cicadas and phytophagous aphids. The

analysis of the values related to the intensity and degree of damage were established in the following sequence reflected in Figure 3, where the curve of the comparative values of the detected diseases according to the parameters of frequency and intensity of the damage is estimated. According to these indices, downy mildew and powdery mildew stand out, followed by *Septoria* leaf spot *Septoria ascophylli* and *Ascochyta* blight *Ascochyta fagopyri* (Figure 3). So, we can mention the consequent damage caused by the above-mentioned diseases, which have a more severe impact on the plants both in the first and in the following growing seasons, annually and

seasonally. Other detected diseases are grey and white mould, root and stem rot. Viral diseases are frequently associated with other diseases, but occur more rarely, therefore, they are considered as facultative diseases that also infest Sakhalin knotweed, with a lower agro-economic importance.

Resulting from the great regenerative capacities of the plant, the affected leaves detach from the stems and the plants continuously generate new structures throughout the growing season, possessing particular tolerance and resistance to the attack of the detected pathogens.

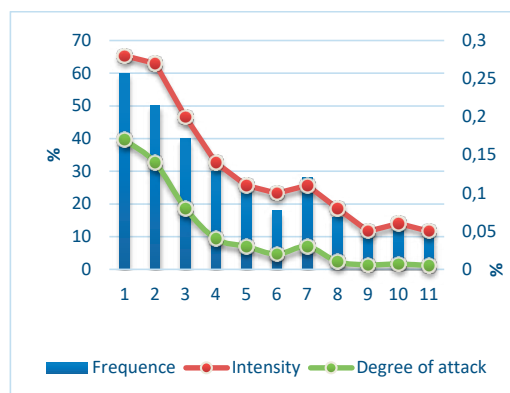


Figure 3. Graphic representation of the phytopathological parameters analysed

Note: 1 - *Perenospora fagopyri*; 2 - *Erysiphe communis*; 3 - *Septoria ascophylli*; 4 - *Ascochyta fagopyri*; 5 - *Phyllosticta polygonorum*; 6 - *Botrytis cinerea* pv. *fagopyri*; 7 - *Sclerotinia sclerotiorum*; 8 - *Fusarium gibbosus*; 9 - *Fusarium oxysporum* f. sp. *spinacia*; 10 - *Cucumis virus*; 11 - *Tobacco mosaic virus (TMV)*

CONCLUSIONS

The species Sakhalin knotweed has acclimatized and adapted very well to the pedoclimatic conditions of the Republic of Moldova, in terms of morpho-structural, physiological aspects and tolerance, in response to the environmental factors of the region, this claim being proven by such features as fast growth, vitality, longevity, regenerative capacity and rich content of valuable biochemical compounds.

As a result of the biological and phytosanitary monitoring, 11 diseases of significant pathological impact, detected for the first time in this species, under the conditions of the Republic of Moldova, were identified, such as:

Perenospora fagopyri Elenev (Berk) de By; *Erysiphe communis* (Wall) Grev. f. *polygoni*; *Septoria ascophylli* Melnic & M. Petrov; *Ascochyta fagopyri* Bres; *Phyllosticta polygonorum* Sacc.; *Botrytis cinerea* Fr., pv. *fagopyri*; *Sclerotinia sclerotiorum* (de By) Korf. Et Dumont.; *Fusarium gibbosus*; *F. oxysporum* f. sp. *spinacia* W.C. Snyder & H.N. Hansen; *Cucumis virus* I Smith; *Tobacco mosaic virus (TMV)*.

The comparative values of the indices of frequency, intensity and degree of damage of pathological interaction with the plants were established individually for each estimated disease and the consecutiveness of the values in descending order is as follows: downy mildew *Perenospora fagopyri* Elenev, with the highest frequency on leaves (60%), followed by powdery mildew *Erysiphe communis* (Wall) Grev. f. *polygoni*, which occurs later, during periods of summer droughts (50%), in the middle of summer, they associate with leaf spot *Septoria ascophylli* Melnic & M. Petrov (40%), *Ascochyta* blight *Ascochyta fagopyri* Bres (30%) and *Phyllosticta* leaf spot *Phyllosticta polygonorum* Sacc. (24%).

However, the species *R. sachalinensis* possesses very high reproduction and regeneration capabilities, especially its foliage. Thus, the affected leaves detach from the plant and it continues to grow new ones, being characterized by tolerance, resistance, high degree of adaptability and ability to maintain high productivity despite the attack of the detected pathogens.

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