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# Increasing the Biodiversity of the Dendroflora of Sparsely Wooded Regions by Adapted Representatives of the Genus *Robinia* L.

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**Abstract:** Purpose of the study: to substantiate the prospects of using various species, varieties and forms of the genus *Robinia* in protective afforestation and landscaping of settlements in dry-steppe and semi-desert zones of southern Russia. It is established that the main limiting factors affecting the growth, development and condition of representatives of the genus *Robinia* in the Volgograd region are winter temperatures up to −37 °C, as well as poor moisture availability and uneven distribution of precipitation during the growing season in combination with extremely low temperatures in the autumn–winter period. All representatives of the generic complex belong to the group of plants that start late and finish the vegetative period late. Phenological atypicity is in the lower half of the normal range, with indicators from +1 °C to 0, which indicates a high level of adaptation to the climatic conditions of the region. In the process of long-term acclimatization, many introduced species have developed a number of genotypic adaptations and are currently able to tolerate extreme winter temperatures up to −37 °C. An assessment of potential drought resistance based on the water-retaining ability of the leaves showed that higher rates of water-resistance capacity (76.8% water loss) are typical of *R. neomexicana*, which has a natural distribution area in the western arid part of the North American continent. *R. viscosa* var. *hartwegii*, with a natural distribution in the areas of the monsoon subtropical climate of eastern North America, is distinguished by low indicators (94.1% water loss), as are clonal decorative forms of *R. pseudoacacia*: f. *pyramidalis* and f. *umbraculifera* (97.6–95.8% water loss), which are common only in culture and characterized by a whole complex of low indicators of bioecological stability. The data obtained by us allow us to recommend the following assortment of species and forms of *Robinia* for protective afforestation and landscaping in the arid territories of southern Russia: *R. pseudoacacia*, *R. neomexicana* var. *neomexicana*, *R. neomexicana* var. *rusbyi* and *R. pseudoacacia x R. neomexicana*.

**Keywords:** climate change; representatives of the genus *Robinia* L.; introduction; leaf apparatus; water-holding capacity; growth processes; adaptation; longevity; arid region

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## 1. Introduction

The problems of preserving Russian forests, especially in the context of increasing changes in the global climate, have been attracting increasing attention both domestically and internationally in recent decades. Already occurring changes in air temperature, an increase in the number of extreme weather events, the risk of forest fires and the possibility of the beginning of active destruction of permafrost can have a catastrophic impact on Russian forests, which account for 21% of all forest plantations on the planet [1–3]. The severe natural conditions of the arid region of the European part of Russia cause an unsatisfactory state of protective plantings, of which there are currently about 500 thousand hectares. In this regard, the problem of increasing their stability and durability is very relevant. The way to solve the problem at the present stage is through the development and

application of a complex of environmental, biological, technological and organizational measures. Their main components are the improvement of the assortment of trees and shrubs used for afforestation in arid conditions; differentiation of afforestation depending on the forest suitability of soils; organization of a seed base based on the selection of the most resistant species; use of different forms and hybrids of trees and shrubs; and improvement of the quality of the used planting material.

It is noteworthy that the effect of various factors of influence, especially meteorological factors, on the growth of forest plantations is not very clear. Therefore, an assessment of the factors affecting the growth of tree and shrub plantations, in particular the effects of climate change, is of great importance for overcoming global changes and expanding forest resources [4]. In the difficult conditions of the dry steppes and semi-deserts of the south-eastern European part of Russia, afforestation has become possible; currently, there are 170 thousand hectares of man-made forests in the Forest Fund of the Volgograd region. The Volgograd region belongs to a sparsely wooded region; it has the richest forestlands in the Lower Volga region, and in terms of forest area, 680.8 thousand hectares in the Southern Federal District is second only to the Krasnodar Territory [5]. The basis for the preservation and multiplication of forest plantations in the region is the reproduction of forests. If we take into account the new forest cover that has arisen on abandoned agricultural lands, but has thus far been ignored by the authorities, the area of forests in Russia is increasing. At the same time, negative changes in their species composition are noted. Due to fires, logging, exposure to diseases and pests, the areas of typical coniferous plantations (spruce, pine) are somewhat reduced, and these species are replaced by secondary species, namely birch and aspen.

In order to create sustainable, long-lasting landscape plantings in difficult forest-growing conditions of the Volgograd region, it is necessary to mobilize new promising species, varieties and forms of woody plants. The genus *Robinia* L. is of great practical and scientific interest in this regard. Currently, only one species has become widespread: *Robinia pseudoacacia*. The remaining representatives of the generic complex are used much less frequently, although an analysis of their bioecological and decorative features indicates that they are not only not inferior, but in fact superior to *R. pseudoacacia* in many respects. The selection and adaptation of representatives of the ancestral complex will provide new opportunities to increase the biodiversity, as well as the productivity, durability and the environmental role of protective strips and decorative landscaping tree plantations [6–12].

*Robinia pseudoacacia* is a well-known forest species native to eastern North America, from where it has been widely introduced worldwide [13]. The species was introduced to Europe in the early 17th century and currently has a significant distribution from the sub-Mediterranean to the warm continental climate [14,15]. *Robinia pseudoacacia* L. is widely distributed in protective forest stands in the south of Russia. Of scientific interest are plantings of the genus *Robinia* in the dry-steppe zone to combat soil degradation and desertification. These species can also be used as low-fertility crops, in protective belts and for decorative purposes with a high degree of drought resistance, as well as in forest restoration given their relatively easy degree of reproduction by seed. Moreover, *Robinia* species are valued as honey-bearing and nitrogen-fixing species, and their wood has several practical applications, including as poles, sleepers, timber, furniture, floor coverings, decorative items and various finishing materials. *Robinia* species are resistant to maximum high or low temperatures and to frequent fluctuations from negative to positive temperatures. The main disadvantage of *Robinia pseudoacacia* L. is low frost resistance [16,17].

The purpose of this study was to substantiate the prospects of using various species, forms and varieties of the genus *Robinia* in protective afforestation and landscaping of settlements in dry-steppe and semi-desert zones of southern Russia.

## 2. Materials and Methods

### *2.1. Plant Resources*

Species, varieties and forms of the genus *Robinia* L., growing in cluster dendrological collections of the Federal Research Center of Agroecology of the Russian Academy of

Sciences, were used in the landscaping of settlements in the Volgograd region and in protective forest plantations. The study was conducted from 2017 to 2022 (Table 1).

**Table 1.** The same-age, introduced populations of representatives of the genus *Robinia* in the landscaping plantings of the Volgograd region.

| Representatives | Type of Landings, Number | Age (Years) | Height (m) | Diameter (mm) | Multi-Barrel Index | Crown Projection (m) |
|---|---|---|---|---|---|---|
| *Robinia neomexicana* var. *neomexicana* Landscaping plantings of the territory of the VolSU (Volgograd: 48.641656° N 44.431266° E). | multi-row | 20 | 5.93 σ 0.28 | 57.1 σ 18.6 | 2.8 σ 0.84 | 4.04 × 4.72 σ 0.55–0.41 |
| *Robinia neomexicana* f. light purple Nursery of Woody Plants of the Federal Research Center of Agroecology of the Russian Academy of Sciences (Volgograd, Sovetsky district 48.631616° N 44.423020° E). | group | 20 | 7.51 σ 0.58 | 70.0 σ 27.5 | 2.5 σ 0.52 | 4.54 × 5.48 σ 1.46–1.53 |
| *Robinia neomexicana* f. light pink Nursery of Woody Plants of the Federal Research Center of Agroecology of the Russian Academy of Sciences (Volgograd, Sovetsky district, 48.631616° N 44.423020° E). | group | 20 | 5.42 σ 0.38 | 55.2 σ 20.5 | 2.4 σ 0.32 | 3.92 × 5.35 σ 0.42–0.71 |
| *Robinia neomexicana* var. *rusbyi* Landscaping plantings of limited and general use (Volgograd: 48.657598° N 44.438422° E). | group | 20 | 7.85 σ 0.75 | 75.2 σ 19.4 | 1.9 σ 0.24 | 4.75 × 5.72 σ 0.45–0.63 |
| *Robinia pseudoacacia* Nursery of Woody Plants of the Federal Research Center of Agroecology of the Russian Academy of Sciences (Volgograd, Sovetsky district, 48.631616° N 44.423020° E). | group | 20 | 10.85 σ 0.69 | 82.9 σ 39.9 | 1.75 σ 1.83 | 5.35 × 6.15 σ 0.53–0.83 |
| *Robinia pseudoacacia* f. *pyramidalis* Forest seed collection plantations on the territory of the Kirov forestry of the Volgograd Forestry (Volgograd, Kirovsky district 48.617162° N 44.373890° E). | row | 20 | 9.04 σ 0.72 | 69.0 σ 33.2 | 5.2 σ 1.54 | 2.4 × 2.6 σ 1.09–1.72 |
| *Robinia viscosa* var. *hartwegii* Landscaping objects of the Central district of Volgograd, limited-use plantings of residential development (Volgograd, 48.714435° N 44.522379° E) | group | 20 | 5.25 σ 1.13 | 144.0 σ 25.1 | 1 σ 0.00 | 3.52 × 4.28 σ 0.58–0.59 |
| *Robinia pseudoacacia x Robinia neomexicana* Nursery of Woody Plants of the Federal Research Center of Agroecology of the Russian Academy of Sciences (Volgograd, Sovetsky district, 48.631616° N 44.423020° E). | group | 20 | 9.25 σ 1.15 | 79.1 σ 24.5 | 1.8 σ 1.52 | 5.15 × 6.05 σ 0.83–1.45 |

To measure the taxation specifications of growing stock, same-age, introduced populations of representatives of the genus *Robinia* were determined.

The taxation specifications of growing stock were determined by the method of mass observations using arithmetic averages. The total number of individual trees studied was 1150.

A survey of the plantings for taxonomic identification was carried out by the route-expedition method. Landscaping and forest protection plantings in various districts of the Volgograd region and the city of Volgograd were subject to the survey. The nomenclature of registered species, varieties and forms of plants was verified according to the system proposed by Peabody [18].

### 2.2. Location of the Experimental Site and Conditions

The research objects are located in typical conditions for the region. The soils on the territory of the nursery of the Federal Research Center of Agroecology of the Russian

Academy of Sciences are light chestnut, medium loamy and medium-thick, formed on a deluvial sand deposit lying at a depth of one meter. The content of mobile forms of nitrogen, phosphorus and potassium is typical for light chestnut soils. The depth of groundwater is 4.0–5.0 m. The humus content is 0.8–1.2%. The collection plantations of the Kirov forestry of the Volgograd Forestry are established on light chestnut, medium loamy soils with a humus content of 1.2–1.5%.

On the territory of the nursery of woody plants of the Lower Volga station for the selection of forest crops of the Federal Research Center of Agroecology of the Russian Academy of Sciences (Kamyshin), the soils are more or less homogeneous, dark chestnut, light loamy and slightly saline, with a humus content of 1.5–2.5%, N–0.22%, $P_2O_5$–0.17% and $K_2O$–1.3%.

In general, the average annual temperatures during the research period ranged from 8.9 °C in 2018 to 9.9 °C in 2021 (Figure 1).

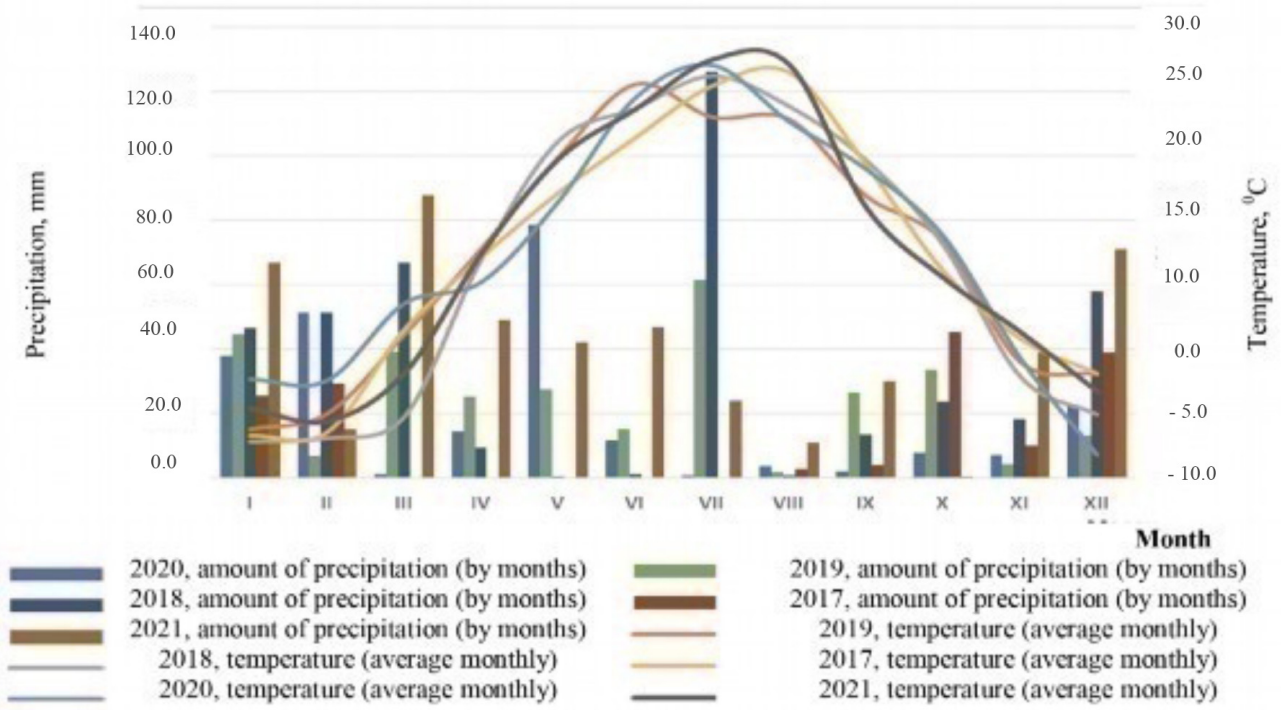



**Figure 1.** Climatogram of the research period (according to the weather station of Volgograd).

These indicators are within two standard deviations (σ = 1.24) from the average annual temperature (8.3 °C) calculated from long-term data for the entire period of observations at the Volgograd weather station, and within one standard deviation (σ = 1.03) from the average annual temperature (8.9 °C) calculated from data for the last 30 years of meteorological observations. In both cases, deviations from the norm are observed towards an increase in average annual temperatures.

The graph constructed for the entire period of meteorological observations from 1944 to 2021 illustrates these processes well (Figure 2). The trend line of this chart is calculated using Equation (1):

$$y = 0.0353\ (x - 1943) + 6.8778 \quad (1)$$

where x is the analyzed year, and y is the predicted average annual temperature.

In accordance with the general trends towards climate warming calculated over a 76-year period of meteorological observations, the climatic norm of average annual temperatures for the city of Volgograd was 9.49 °C in 2017 and 9.80 °C in 2021.

Calculations show that the average annual temperatures in 2017–2021 were close to the trend line and even fell below the average; consequently, the temperature regime of

the research period can be characterized as typical for a region with a general tendency to increase average annual temperatures.

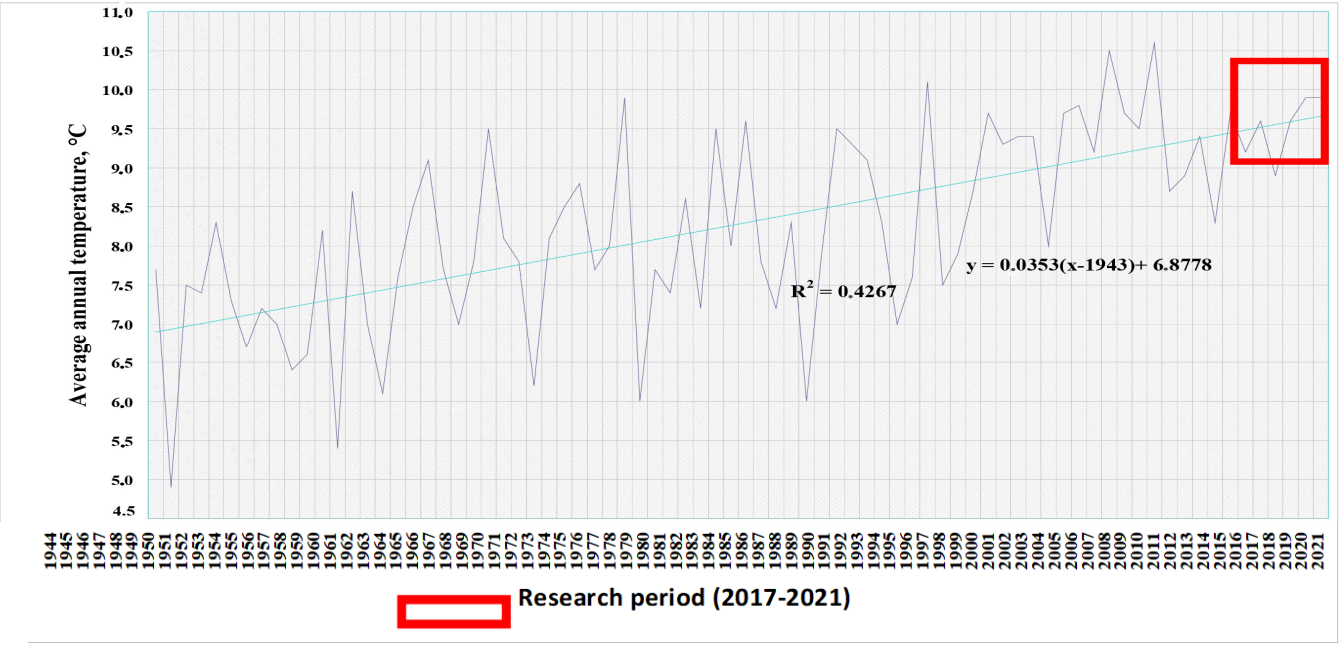



**Figure 2.** Average annual temperatures according to the Volgograd weather station for 1944–2021. The red box highlights the research period (2017–2021).

Agro-climatic conditions in various agroforestry and administrative districts of the Volgograd region have different degrees of similarity to natural conditions in the areas of natural distribution of species in North America. Cluster analysis based on the calculation of Euclidean distances made it possible to group points by similarity of climatic characteristics.

The comparison used meteorological indicators from US states with spatial distribution of certain species of the genus *Robinia* [19], with meteorological indicators of various districts of the Volgograd region [20]. The calculations took into account the average temperature of the coldest month, the average annual precipitation and the average annual temperature. The dendrogram of the similarity of climatic characteristics is shown in Figure 3.

### *2.3. Maintaining the Prototype, Records and Observations*

The evaluation of the prospects of the studied species, varieties and forms was carried out according to the methodology of the All-Russian Research Institute of Agroforestry [21]. To analyze biological stability (winter hardiness, drought resistance, reproductive ability, vitality), we used data from our own observations, as well as data from long-term visual observations conducted on the basis of cluster dendrological collections of the Federal Research Center of Agroecology of the Russian Academy of Sciences [22]. The study of drought resistance was carried out according to visual observations in natural conditions, as well as by an indirect method for the water-retaining ability of leaves during the growing season (June–August), expressed as a percentage of the ratio of lost water to its primary content. The material for analysis (leaves from the annual increments of the middle part of the plant) was collected in the morning and quickly transported to the laboratory to determine the water retention capacity by the weight method according to Equation (2):

$$\mathrm{WRA} = \frac{B - b}{A} \times 100 \quad (2)$$

where $B$ is the initial mass, mg; $b$ is the weight after withering (after 1, 2, 3, 4, 5, 6, 24 h), mg; and $A$ is the initial water content in the leaves, mg.

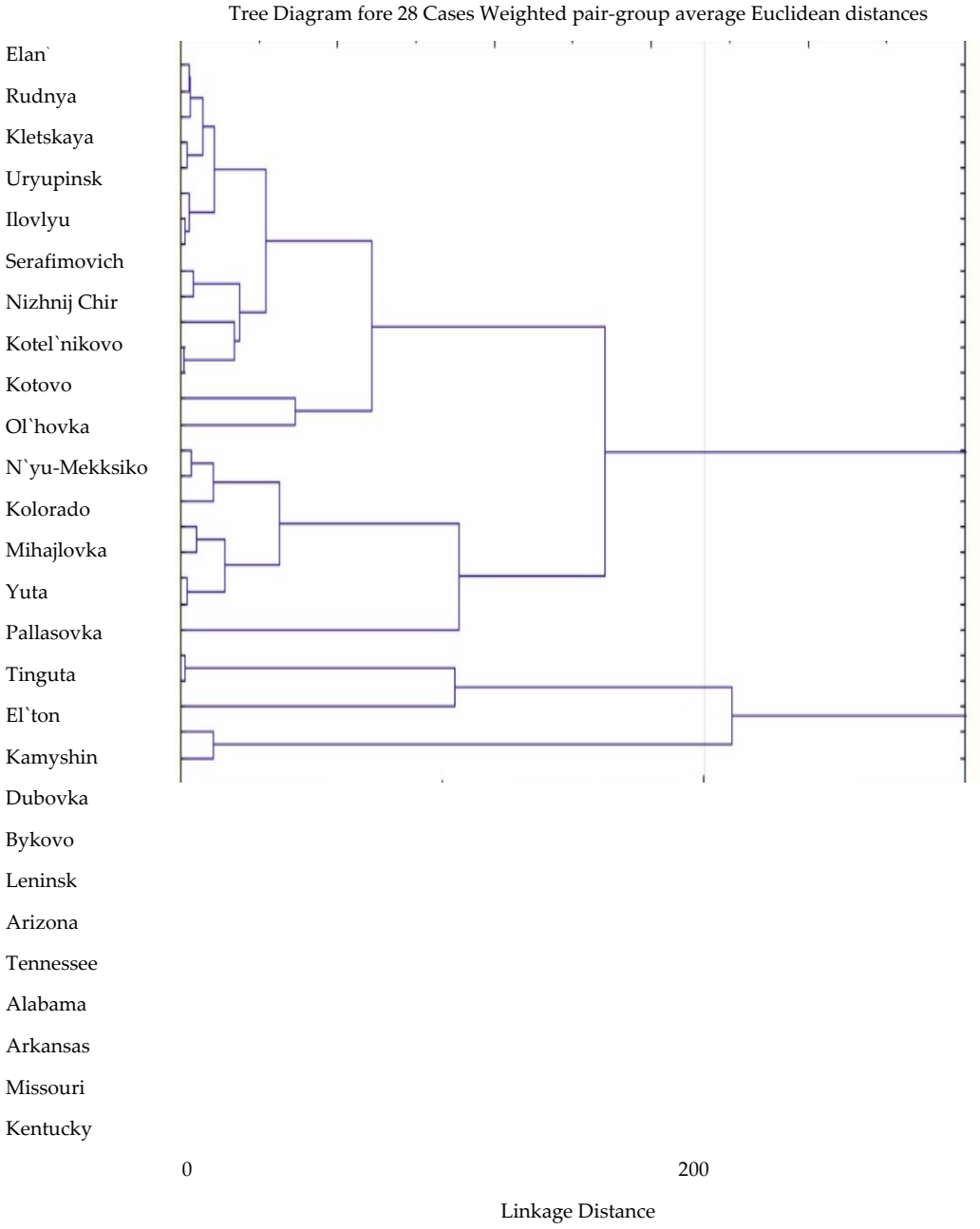



**Figure 3.** Dendrogram of similarity of climatic indicators of natural and secondary distribution areas of species of the genus *Robinia* L.

At the same time, the content of pigments in the leaves of trees was determined. To do this, 10 leaf samples were taken from each tree. To measure the content of chlorophyll, flavonoids, anthocyanins and the nitrogen balance index (NBI) in the epidermis of leaves of woody plants, a "Dualex Scientific+" device ("Force-A", France) was used. The nitrogen balance index (NBI) shows the ratio of the amount of chlorophylls and flavonoids (nitrogen/carbon) and is presented in conventional units.

Laboratory analysis was performed in the Laboratory of Bioecology of Woody Plants of the Federal Research Center of Agroecology of the Russian Academy of Sciences. All samples were dried at the same temperature (20–23 °C), which was recorded by thermometers, under the same conditions of illumination and humidity (one laboratory), using the same containers for drying each sample and other equal conditions for each sample. Weighing was carried out on electronic laboratory scales (VK-600) with an accuracy of 0.001 g.

The habitat conditions and the state of the forest stand were studied. In the absence of direct or weak indicators of frost resistance, indirect phenological and morphological features of *Robinia pseudoacacia* were taken into account, which should be decisive if several signs are equal.

A 3-point scale was used to assess the vitality of plants. The winter hardiness of plants was assessed on a 6-point Pyatnitsky scale and a 7-point scale developed by the Main Botanical Garden of the Academy of Sciences (MBG RAS): I—the plant does not freeze; II—freezes no more than 50% of the length of annual shoots; III—freezes from 50 to 100% of the length of annual shoots; IV—freezes not only annual, but also older shoots; V—the aboveground part freezes to the snow cover; VI—the entire aboveground part freezes; VII—the plant freezes completely. Potential frost resistance was studied by freezing shoots in a climatic chamber (KHT-0.22). To assess the frost resistance of tree species, the freezing index was determined and the data obtained were compared with the following scale of the MBG RAS: 0—the plant does not freeze; 0.1–0.9—the plant freezes slightly; 1.0–7.9—the plant freezes moderately; 8.0–69.9—the plant freezes significantly; 70.0–100.0—the plant freezes completely. Here, 0–100.0 are the values of the freezing index, in % [23].

Reproductive ability was studied on a 5-point scale. The periodicity, duration and intensity of flowering, yield, entry into the generative phase of development, seed quality, weight of 1000 seeds, germination and germination energy were taken into account. According to the monitoring and mapping of introduced populations, a comprehensive assessment of invasive activity was carried out and conclusions were made based on meta-analysis. The status of invasive activity is assigned to alien species, according to the classification adopted in the project of European botanical gardens: 1—alien species that are massively widespread both on the territory of dendrological collections and beyond; 2—species that actively settle on the territory of the arboretum that is not occupied by collections and expositions; 3—species that have formed local naturalizing populations outside collections or expositions, and in the case of vegetative overgrowth—stable clones, those that have lost their physical connection with the mother plants; 4—species marked at least once outside the number of sites [24,25].

The prediction of the adaptive capabilities of introduced representatives of the genus *Robinia* was carried out by the method of climatic analogues, the main provisions of which were formulated in the works of Mayr and subsequently refined by Pavari, Selyaninov, Maleev and Guda [22]. The climatic conditions of the regions of natural growth of the studied species and the areas of introduction were compared according to the following indicators: absolute minimum and absolute maximum air temperature, average annual temperatures and average annual precipitation. To identify patterns, multivariate analysis was used by the method of hierarchical clustering with the construction of dendrograms.

The taxation specifications of plantings were determined by the method of mass observations using arithmetic mean values and standard deviation (σ). For the taxational characteristics of the life form and the general habitus of plants, the authors additionally proposed a multi-stem index—the average number of trunks per plant in the sample population.

The state of the nodule structure was determined by Keller light microscopy. A microtome was used to obtain a cross section up to 8 microns thick from a root nodule embedded in paraffin.

### *2.4. Statistical Analysis*

Statistical processing of the obtained data was carried out according to the method of Zaitsev [26]. Mathematical processing of the results was carried out in the MS Excel application program using standard algorithms: arithmetic mean with absolute and relative errors (significance of differences between individual indicators), coefficient of variation and standard deviation. The main statistical data were calculated by the method of dispersion analysis of data. Classification analysis was carried out by the method of hierarchical clustering using the computer programs “Statistica” and “MS Excel”.

## 3. Results

The analysis of the similarity of the climatic indicators of the donor regions and the points of introduction allowed us to determine that *Robinia neomexicana*, growing in the states of Colorado, New Mexico, Arizona and Utah, has an advantage over other species (Figure 3). It should be noted that the meteorological conditions of these states have the maximum similarity with the Volgograd region both in terms of temperature indicators and the total amount of precipitation [27–36].

A comprehensive assessment of the prospects in terms of vitality, winter hardiness, drought resistance and reproductive ability, including the features of flowering and fruiting [37], allowed us to identify typical representatives of *Robinia pseudoacacia* and its interspecific hybrids with *Robinia neomexicana* with maximum reproductive ability (4.5–4.6 points) (Table 2). According to a 3-point scale of vitality, all representatives in the dry-steppe conditions of the Volgograd region are in the range from 1.2 to 2.7 points. Relatively low vitality is observed in the decorative form of *Robinia pseudoacacia* f. *pyramidalis* (2.5–2.7 points). The remaining representatives are characterized by high indicators of vitality from 1.2 to 1.5 points.

**Table 2.** Comprehensive assessment of the indicators of the prospects of introduction according to visual observations.

| Plant | Winter Hardiness [1] | Drought Resistance [2] | Blossom [3] | Fruiting [4] | Vitality [5] |
|---|---|---|---|---|---|
| *R. pseudoacacia* L. | 1.8<br>m [6] = 0.043 | 1.1<br>m = 0.034 | 4.7<br>m = 0.052 | 4.6<br>m = 0.056 | 1.2<br>m = 0.046 |
| *R. pseudoacacia* f. *pyramidalis* | 3.7<br>m = 0.049 | 1.2<br>m = 0.042 | 0<br>m = 0 | 0<br>m = 0 | 2.7<br>m = 0.082 |
| *R. neomexicana* var. *neomexicana* | 1.2<br>m = 0.048 | 1.5<br>m = 0.055 | 4.5<br>m = 0.057 | 4.2<br>m = 0.046 | 1.3<br>m = 0.048 |
| *R. neomexicana* var. *rusbyi* | 1.3<br>m = 0.051 | 1.6<br>m = 0.053 | 4.6<br>m = 0.056 | 4.3<br>m = 0.052 | 1.3<br>m = 0.051 |
| *R. neomexicana* f. light violet | 1.7<br>m = 0.049 | 1.3<br>m = 0.052 | 3.7<br>m = 0.052 | 3.5<br>m = 0.055 | 1.4<br>m = 0.056 |
| *R. neomexicana* f. light pink | 1.5<br>m = 0.055 | 1.4<br>m = 0.054 | 3.9<br>m = 0.057 | 3.7<br>m = 0.051 | 1.5<br>m = 0.060 |
| *R. viscosa* var. *hartwegii* | 2.2<br>m = 0.086 | 1.3<br>m = 0.051 | 4.9<br>m = 0.050 | 1.7<br>m = 0.051 | 1.5<br>m = 0.062 |
| *R.pseudoacacia x R. neomexicana* | 1.5<br>m = 0.055 | 1.1<br>m = 0.038 | 4.5<br>m = 0.055 | 4.5<br>m = 0.057 | 1.2<br>m = 0.043 |

[1] 1—The plant is not damaged by winter conditions; 2—Annual shoots are occasionally damaged by up to 50%; 3—Shoots of the current year completely die off and older branches are partially damaged; 4—2–3-year-old shoots and skeletal branches freeze; 5—The aboveground part of plants completely dies (or above the level of snow cover); 6—Plants freeze completely; [2] 1—The plant does not react to arid conditions; 2—The turgor of foliage and needles is lowered, and plants noticeably reduce growth in this or the following year; 3—Needles, foliage and young shoots are damaged; the growth of this or the following year is reduced many times or is absent altogether; 4—Skeletal branches are damaged, and the plant does not restore its size in the following year; 5—The entire aboveground part of the plant dies; 6—The plant dies completely from drought; [3, 4] 0—there is no flowering and no harvest; 1—very weak flowering or very poor harvest; 2—weak flowering and weak harvest (fairly satisfactory and uniform flowering or fruiting—on free-standing trees and on trees growing along the edges, sometimes in plantings); 3—average flowering or average yield (quite significant flowering or fruiting—on free-standing trees, on trees growing along the edges; satisfactory flowering and fruiting in medium-aged and ripe plantings); 4—good flowering or good harvest (abundant flowering or fruiting—on free-standing trees and on trees growing on the edges; good flowering or fruiting—in medium-aged and ripe plantings); 5—very good flowering or very good harvest (abundant flowering or fruiting—on trees growing on the edges, freely and in middle-aged and ripe plantings); [5] 1—good vitality—the plant is well developed, has a healthy appearance, well-developed shoots, buds and leaves, normal color, blooms abundantly or well and bears fruit; 2—satisfactory or average vitality—the overall development of the plant is somewhat weaker; the growth of shoots, leafing, flowering and fruiting does not reach a maximum; 3—weak vitality—the plant is noticeably weakened, the growth of shoots is insignificant, flowering and fruiting are single or absent; [6]—arithmetic mean error.

Despite the relatively high degree of biological resistance, plants of this group have different potential winter hardiness. Thus, for the northern steppe regions, the interspecific hybrids *R. pseudoacacia x R. neomexicana* are more promising in comparison with *R. pseudoacacia*. *R. viscosa* also has a relatively low winter hardiness. This feature, along with low reproductive properties, allows us to recommend *R. viscosa* as an additional

species useful in the southern regions but with limited use in the northern regions of the Volgograd region.

As our studies have shown, in the vegetatively propagated form of *Robinia pseudoacacia* f. *pyramidalis* (Pepin) Rehd. there is still an annual freezing of shoots by 25–50% of the length of the previous year's growth (Figure 4).



**Figure 4.** Annual freezing of shoots of *Robinia pseudoacacia* f. *pyramidalis* by 25–50% of the length of annual growth (Volgograd, seed plantations of the Federal Research Center of Agroecology of the Russian Academy of Sciences). Red arrows mean dry shoots as a result of freezing.

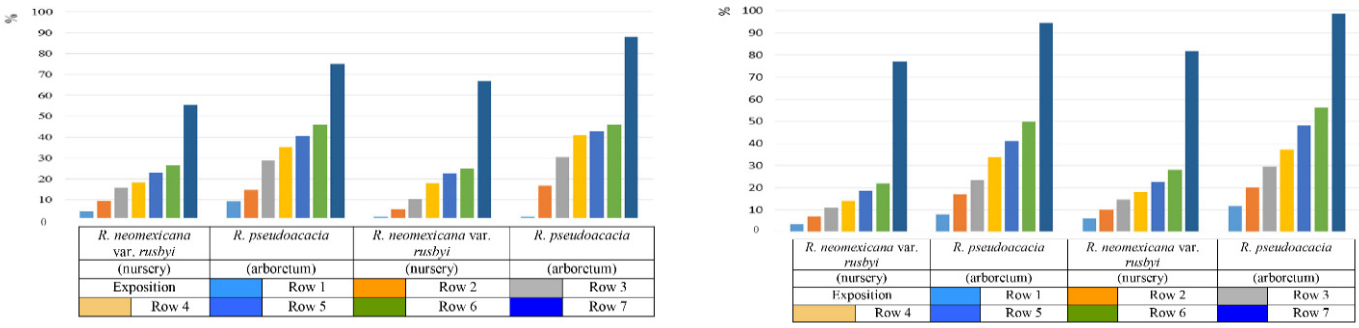
A series of unfavorable conditions in 2017–2022 had a destructive effect on the viability of various representatives of the genus *Robinia*. One of the main damaging factors during this period were the low average monthly temperatures and their distribution during the winter period. In December, the average monthly temperature was −7.9 °C, which is 3.3 °C lower than the climatic norm calculated for the entire period of observations at the weather station of Volgograd. According to the results of annual visual observations in the spring of 2021, the lowest winter hardiness was recorded during the research period from 2017 to 2022.

Despite periodic damage in severe winters, most representatives of the genus *Robinia* in the conditions of the Volgograd region have good long-term average winter hardiness. The most thermophilic was the decorative form of *Robinia pseudoacacia pyramidalis*, the annual shoots of which freeze annually. In some years, two-year-old shoots or more than half the length of the annual growth were damaged. All forms and typical representatives of *Robinia neomexicana*, as well as hybrid forms of *R. pseudoacacia x R. neomexicana*, turned out to be more winter hardy. Insufficient winter hardiness of decorative forms of *Robinia pseudoacacia* is explained by the fact that their reproduction in culture occurs mainly in a vegetative manner, which does not allow them to adapt to changing environmental conditions [38].

The potential winter hardiness of representatives of the genus *Robinia* was also confirmed by the data of the in-house study of frost resistance by freezing shoots in the climatic chamber KHT-0.22. Studies showed that exposure to a temperature of −37 °C for 24 h does not cause significant damage to shoots in typical representatives of *R. neomexicana* Gray, *R. pseudoacacia* L.; *Robinia viscosa* Vent. var. *hartwegii* and (Kene) ash.

The study of drought resistance was carried out according to visual observations in full-scale conditions, as well as by an indirect method for the water-retaining ability of leaves. It has been established that the water-retaining ability of the leaves of the genus *Robinia* is significantly influenced by the cultivation conditions [39–48]. Comparative characteristics of the water-retaining ability of the leaves of *R. pseudoacacia* and *R. neomexicana* var. *rusbyi* allowed us to determine that the best indicators of the water regime are observed in plants

growing in the nursery of woody plants of the Federal Research Center of Agroecology of the Russian Academy of Sciences. This feature is associated with the cultivation conditions, according to which the plantings on the territory of the nursery have a better moisture supply (Figure 5a).




(**a**) (**b**)

**Figure 5.** Water–holding capacity of leaf tissues ((**a**)—July, (**b**)—September, on average 2019–2021) of *R. pseudoacacia* and *R. neomexicana* var. *rusbyi* under various cultivation conditions, expressed in % ratio of lost water to the original content.

By the beginning of September, against the background of a general decrease in water-retention capacity, plants growing in different conditions maintain a difference in the values of water-retention capacity (Figure 5b).

Comparison of water-retention capacity indicators during the growing season with the main climatic characteristics of the research period showed that the fastest water loss was observed during prolonged atmospheric and soil droughts. In accordance with the main meteorological indicators of the Volgograd region, the average monthly precipitation in the summer period decreased from June to August, which led to a deterioration in the conditions of moisture supply and a decrease in the water-holding capacity of the leaves (Table 3).

A comparative analysis of the water-holding capacity of various species of the genus *Robinia* revealed the following patterns. Regardless of the time of the study, the maximum rates of water loss were observed in the decorative form of *R. pseudoacacia* f. *pyramidalis*. *R. viscosa* var. *hartwegii* is also distinguished by its low water-holding capacity.

With an increase in the air temperature, the magnitude of the real water deficit in the leaves increases. All representatives of the genus *Robinia* are very drought-resistant. Comparison of the indicators of the water-holding capacity of the leaves with the data of observations obtained under field conditions allowed us to identify a number of adaptations to the effects of prolonged summer atmospheric and soil drought:

- rhythmic nictinastic movements of the leaflets on a complex leaf—this mechanism is active and is not associated with the general loss of leaf turgor during exposure to high temperatures (Figure 6);
- summer leaf fall is a decrease in the number of leaves without reducing the overall vitality of the plant [49]. Leaf fall in *R. neomexicana* and its forms is observed in the second–third decade of July; in *R. pseudoacacia*, this occurs during the third decade of July through the first decade of August.

Comparison of visual observation data with indirect assessment methods indicates the presence of an inverse relationship between the beginning of summer leaf fall and water-retention capacity. The earliest summer leaf fall begins in *R. neomexicana* with high water-retention capacity.

Thus, the differences in the water-holding capacity of the leaves of representatives of the genus *Robinia* are relatively constant and change only slightly over the years.

A reaction to the cultivation conditions of *R. pseudoacacia* and *R. neomexicana* var. *rusbyi* was also noted at the physiological level in the functioning of the photosynthetic apparatus (Table 4).

**Table 3.** The water-retaining ability of the leaf tissues of representatives of the genus *Robinia*, expressed in % ratio of lost water to the initial content after exposure for 24 h.

| Species, Varieties and Forms of the Genus *Robinia* | Term of Determination | 2019 | | 2020 | | 2021 | | Average |
|---|---|---|---|---|---|---|---|---|
| | | Water-Holding Capacity, % | HTC * | Water-Holding Capacity, % | HTC * | Water-Holding Capacity, % | HTC * | |
| *R. pseudoacacia* f. *pyramidalis* | July | 93.0 | 1.03 | 97.7 | 1.16 | 98.5 | 0.28 | 96.4 σ = 2.97 |
| | September | 94.2 | 0.60 | 98.9 | 0.01 | 99.6 | 0.70 | 97.6 y = 2.93 |
| *R. viscosa* var. *hartwegii* | July | 86.2 | 1.03 | 90.6 | 1.16 | 93.7 | 0.28 | 90.2 σ = 3.77 |
| | September | 89.2 | 0.60 | 97.1 | 0.01 | 96.0 | 0.70 | 94.1 y = 4.28 |
| *R. pseudoacacia x R. neomexicana* | July | 81.6 | 1.03 | 84.7 | 1.16 | 89.8 | 0.28 | 85.4 σ = 4.13 |
| | September | 87.0 | 0.60 | 90.5 | 0.01 | 95.7 | 0.70 | 91.1 y = 4.40 |
| *R. pseudoacacia* | July | 78.9 | 1.03 | 81.7 | 1.16 | 88.0 | 0.28 | 82.9 σ = 4.68 |
| | September | 89.8 | 0.60 | 93.0 | 0.01 | 98.7 | 0.70 | 93.8 y = 4.49 |
| *R. neomexicana* f. light violet | July | 67.7 | 1.03 | 71.8 | 1.16 | 75.7 | 0.28 | 71.7 σ = 4.00 |
| | September | 78.4 | 0.60 | 83.2 | 0.01 | 87.6 | 0.70 | 83.1 y = 4.58 |
| *R. neomexicana* f. light pink | July | 62.3 | 1.03 | 67.6 | 1.16 | 69.0 | 0.28 | 66.3 σ = 3.55 |
| | September | 79.4 | 0.60 | 86.1 | 0.01 | 87.8 | 0.70 | 84.5 y = 4.46 |
| *R. neomexicana* var. *rusbyi* | July | 58.6 | 1.03 | 62.9 | 1.16 | 66.8 | 0.28 | 62.8 σ = 4.11 |
| | September | 71.6 | 0.60 | 77.2 | 0.01 | 81.7 | 0.70 | 76.8 y = 5.07 |
| Average by year | July | 77.1 σ = 12.70 | | 81.6 σ = 13.04 | | 84.6 σ = 12.29 | | |
| | September | 85.2 σ = 7.82 | | 90.3 σ = 7.59 | | 93.4 σ = 6.74 | | |

* Selyaninov hydrothermal moisture coefficient.



**Figure 6.** Folding of leaflets of a complex leaf of *R. neomexicana* under the influence of high temperature and light.

**Table 4.** The pigment system of *R. pseudoacacia* and *R. neomexicana* var. *rusbyi* in various cultivation conditions, mcg/cm$^2$ of raw mass.

| Indicator | *Robinia* Form | | | |
|---|---|---|---|---|
| | ***R. pseudoacacia* (Hatchery)** | ***R. pseudoacacia* (Arboretum)** | ***R. neomexicana* var. *rusbyi* (Hatchery)** | ***R. neomexicana* var. *rusbyi* (Arboretum)** |
| *Chlorophyll* | 39.4 ± 0.7 | 35.5 ± 1.01 | 39.6 ± 0.43 | 34.8 ± 0.99 |
| Flavonoids | 1.85 ± 0.02 | 1.67 ± 0.03 | 1.84 ± 0.02 | 1.53 ± 0.04 |
| Anthocyanins | 0.047 ± 0.002 | 0.050 ± 0.003 | 0.042 ± 0.002 | 0.053 ± 0.003 |
| NBI, units | 21.4 ± 0.4 | 24.8 ± 2.0 | 21.5 ± 0.3 | 24.9 ± 1.5 |

The content of chlorophyll and flavonoids in the leaves of plants growing in the nursery was higher than that of plants in the arboretum, while the content of anthocyanins and the level of the nitrogen balance index (NBI) were lower. An increase in the content of anthocyanins and the NBI in plants growing in the arboretum may be associated with a specific protective reaction to a complex of unfavorable growing conditions.

Methods of light microscopy allowed us to establish the relationship between the conditions of the place of growth and the state of the nodule structure during the growing season (Figure 7).

The nodules are perennial, and new blades are formed annually, developing throughout the growing season. The cross-section shows areas with infected and areas with non-infected cells. Darker infected areas contain a large number of vesicles.

To differentiate the plants of the first cluster into 'leading' and 'additional' members of the recommended assortment, a point assessment of bioecological indicators, as well as the availability of conditions for the mass production of planting material, showed that *R. viscosa* var. *hartwegii* has the lowest yield [49]. Small tree arrays in the conditions of the Volgograd region are represented by mother plantations of a typical variety of *R. neomexicana* var. *neomexicana*, color forms of *R. neomexicana* and hybrids of *R. pseudoacacia x R. neomexicana*. The limited stock of seed material of these plants at this stage of introduction allows us to recommend them for use as an additional assortment. Plants of the second cluster, i.e., *Robinia pseudoacacia* f. *pyramidalis* and *Robinia pseudoacacia* f. *umbraculifera*, have the lowest degree of bioecological stability. These forms reproduce only vegetatively and are characterized by low winter hardiness. Annual shoots freeze every year, and in some years, long-term shoots and more than half of the annual growth are damaged [50].

In accordance with the forest-reclamation zoning of the Volgograd region, it is advisable to recommend these forms for limited use in the southern semi-desert Ergeninsko-Sarpinsky district (Table 5).

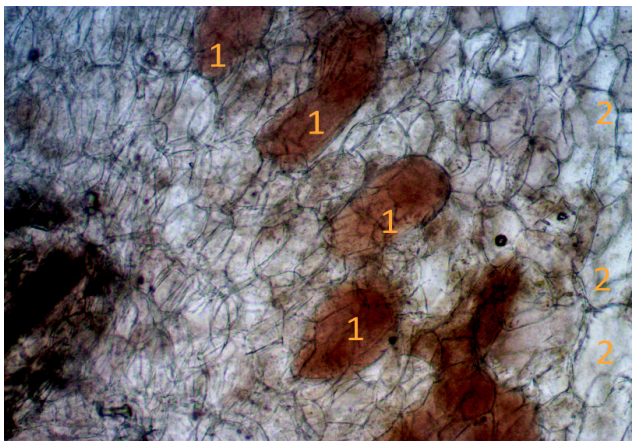



**Figure 7.** Cross section of the root nodule. Note: 1—zone with infected cells; 2—zone with uninfected cells. Scale: 1 cm = 100 microns.

**Table 5.** The prospects of representatives of the generic complex for various agroforestry districts of the Volgograd region.

| Taxon | Volga-Ural Semi-Desert | Volga-Don Steppe | Volga-Ural Dry Steppe | Ergeninsko-Sarpinsky Semi-Desert | Volga-Don Dry Steppe | Status of Invasive Activity * |
|---|---|---|---|---|---|---|
| *R. pseudoacacia* | L/A | A | A | L | L/A | 2.0 |
| *R. pseudoacacia* f. *pyramidalis* | N/LIM | N | N | A | N/LIM | 0 |
| *R. neomexicana* | L | L | L | L | L | 3.0 |
| *R. neomexicana* f. light purple | A | A | A | A | A | 4.0 |
| *R. neomexicana* f. light pink | A | A | A | A | A | 4.0 |
| *R. viscosa* var. *hartwegii* | A/LIM | LIM | LIM | A | LIM/A | 4.0 |
| *R.pseudoacacia x R. neomexicana* | A | A | A | A | A | 3.0 |

L—leader; A—additional; LIM—limited; N—not recommended for the assortment. * 1—alien species that are massively widespread both on the territory of dendrological collections and beyond; 2—species that actively settle on the territory of the arboretum that is not occupied by collections and expositions; 3—species that have formed local naturalizing populations outside collections or expositions, and in the case of vegetative overgrowth-stable clones, those that have lost their physical connection with the mother plants; 4—species marked at least once outside the sites [24,25].

Taking into account the bioecological features of representatives of the generic complex, they can be recommended for different types of forest protection and landscaping plantings (Table 6).

Typical representatives of *Robinia pseudoacacia* and its interspecific hybrids with *Robinia neomexicana*, characterized by high growth rates, are promising for protective plantings. *Robinia neomexicana* is advisable to use in ravine-beam plantings [12].

In garden and park construction, representatives of the generic complex are promising for the creation of irrigated and non-irrigated landscaping facilities of general, limited use and special purpose. The use of the thermophilic form of *Robinia pseudoacacia* f. *pyramidalis* is possible only in landscaping facilities of limited use. To grow these decorative forms, it is necessary to choose windproof areas with southern exposure. *Robinia glutinosa*, as the most decorative species of the genus *Robinia*, is advisable to use not only in groups, but also in solitary plantings.

**Table 6.** Assortment of *Robinia* species and forms suitable for various types of protective afforestation and landscaping.

| Name of the Plant | Protective Forest Plantations | | Landscaping | | | |
|---|---|---|---|---|---|---|
| | Protective | Gully-Girders | Arrays | Groups | Solitaire | Alleys |
| *R. pseudoacacia* | Y | Y | Y | Y | | Y |
| *R. pseudoacacia* f. *pyramidalis* | | | | | | Y |
| *R. neomexicana* var. *neomexicana* | | Y | Y | Y | | |
| *R. neomexicana* var. *rusbyi* | | Y | Y | Y | | |
| *R. neomexicana* f. light purple | | | | Y | | |
| *R. neomexicana* f. light pink | | | | Y | | |
| *R. viscosa* var. *hartwegii* | | | | Y | Y | |
| *R.pseudoacacia x R. neomexicana* | Y | Y | | Y | | |

A check mark means belonging to different types of protective afforestation and planting of greenery.

## 4. Discussion

For most species of the genus *Robinia* L., a number of phylogenetic adaptations for growing in certain environmental conditions have developed in the process of evolution:

- anatomical and morphological adaptations to growing in arid regions with a highly branched root system [48] and xeromorphic structure of leaves [51]. The tracery and

wind permeability of *Robinia* crowns is a clear adaptation for growing in regions with increased wind load;

- physiological and biochemical phylogenetic adaptations. *R. pseudoacacia* is able not only to easily tolerate prolonged droughts, reducing transpiration and photosynthesis intensity, but also to recover quickly, compensating for growth processes after exposure to adverse environmental factors [52,53];
- phenotypic ontogenetic adaptations, including changes in life form (tree–shrub, single-stemmed tree–multi-stemmed tree), slowing down the growth rate, reducing the overall size of plants, reducing the size of leaf blades, etc.;
- ecological and phytocenotic phylogenetic adaptations—adaptations that ensure the ability of plants to occupy ecological niches in the biocenosis—the ability to settle and enter plants into the natural ecosystems of the point of introduction (naturalization).
- genotypic adaptations are a comparative characteristic of the winter hardiness of varieties of *R. pseudoacacia*, whose reproduction occurs exclusively by vegetative methods, with typical representatives of this species, whose reproduction occurs mainly by the generative method. The absence of genetic heterogeneity in maple plantations excludes the possibility of natural or artificial selection and the formation of new genotypic adaptations in changing growing conditions. That is why generative reproduction and generational change are a prerequisite for the adaptation of plants in the process of introduction. The most thermophilic species is *R. viscosa*, and the most hardy is *R. neomexicana* (*R. luxurians*). The latter was used in breeding, in order to increase the winter hardiness of *R. pseudoacacia* by hybridization [16]. As our research has shown, at present the difference in winter hardiness of *Robinia glutinosa*, *R. pseudoacacia* and *R. neomexicana* has significantly decreased. Over the last century, in the process of natural and artificial selection, these species have developed a number of genotypic adaptations to low negative temperatures.

Like many plants of the Fabaceae family, all representatives of the genus *Robinia* have symbiotic relationships with nitrogen-fixing bacteria and are therefore able to grow on poor soils, enriching them with nitrogen.

Typical representatives of *R. neomexicana* are recommended for wide introduction into culture (the leading assortment), and color forms of this species as an additional assortment in all agroforestry areas of the Volgograd region.

A broad culture of *R. pseudoacacia* is appropriate in the southern part of the Volga–Don forest-steppe, Volga–Ural semi-desert and in the Ergenin–Sarpin semi-desert areas. In other areas, this type is promising only as an additional assortment.

Low frost resistance and reproductive capacity of *R. viscosa* var. *hartwegii* and decorative forms of *R. pseudoacacia* allow us to attribute them to a limited and additional assortment in various agroforestry areas of the southern part of the Volgograd region.

For the creation of tree massifs in the conditions of the Volgograd region, *R. pseudoacacia* is recommended; for groves: *R. neomexicana* and *R. pseudoacacia x R. neomexicana*; for alleys: *R. pseudoacacia* f. *pyramidalis* and *R. pseudoacacia* f. *umbraculifera*; and for solitary plantings: *R. viscosa* var. *hartwegii*.

## 5. Conclusions

It is established that the main limiting factors affecting the growth, development and condition of representatives of the genus *Robinia* in the Volgograd region are winter temperatures up to −37 °C, as well as poor moisture availability and uneven distribution of precipitation during the growing season in combination with extremely low temperatures in the autumn–winter period. Based on the analysis of the similarity of climatic indicators of donor regions and points of introduction, it was revealed that *R. neomexicana* has an advantage over other species, whose natural distribution area lies in the United States (Utah, New Mexico, Arizona and Colorado).

All representatives of the generic complex belong to the group of plants that start late and finish vegetative growth late. Phenological atypicity is in the lower half of the

normal range with indicators from +1 °C to 0, which indicates a high level of adaptation to the climatic conditions of the region. In the process of long-term acclimatization, many introduced species have developed a number of genotypic adaptations and are currently able to tolerate extreme winter temperatures up to −37 °C.

The studied species have high indicators of viability in difficult growing conditions of the Volgograd region. An assessment of potential drought resistance based on the water-retaining ability of the leaves showed that higher rates of water-resistance capacity (76.8% water loss) are typical for representatives of *R. neomexicana* with a natural distribution area in the western arid part of the North American continent. *R. viscosa* var. *hartwegii*, with a natural distribution in the areas of the monsoon subtropical climate of eastern North America, is distinguished by low indicators (94.1% water loss), as are clonal decorative forms of *R. pseudoacacia:* f. *pyramidalis* and f. *umbraculifera* (97.6–95.8% water loss), which are common only in culture and are characterized by a whole complex of low indicators of bioecological stability.

The arid climatic regime of the Volgograd region imposes special requirements on introduced woody plants in terms of drought resistance and winter hardiness. The data obtained by us allow us to recommend the following assortment of species and forms of *Robinia* for protective afforestation and landscaping in the arid territories of southern Russia: *R. pseudoacacia, R. neomexicana* var. *neomexicana, R. neomexicana* var. *rusbyi* and *R. pseudoacacia x R. neomexicana*.

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