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Seed Germination and Seedling Growth of *Robinia pseudoacacia* Depending on the Origin of Different Geographic Provenances

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Abstract: Black locust (*Robinia pseudoacacia*) is recognised as a forest species of interest due to its multiple uses. The management of forest genetic resources and their efficient conservation suffer from variations in traits and start with seed germination. The aim of the current study was to investigate the germination of seeds obtained from plus trees selected in eight Romanian provenances, as well as to investigate the influence of the origin upon plants' growth and development. Two experiments were undertaken to test seed germination: one treatment involved water-soaked seeds and heat/cold treatment, while the other treatment was based on sulphuric acid, at different concentrations (50, 70, 90%). The results were correlated with the morphological analysis of the seeds. Satu-Mare had the lowest germination rate within both treatments. Sulphuric acid did not improve seed germination as much as the heat treatment. The highest germination rate occurred for the water and temperature treatment on seeds from Bihor provenance (68.2%). The most distant provenance was Bihor, in inverse correlation with Bistrița Năsăud and grouped separately within the hierarchical dendrogram of cluster analysis based on the analysed parameters of the provenances investigated. The results demonstrated that the genotypes and environmental heterogeneity of the seed origin within the provenances may finally result in different performances.

Keywords: black locust; forest; germination; provenances; seed dormancy

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1. Introduction

The *Robinia* genus includes 19 botanical taxa, eight of which are classified as species, while the others are considered natural varieties or hybrids [1,2]. Black locust (*Robinia pseudoacacia* L.) is native to the eastern part of North America—the Appalachian region [3,4]. In Europe, it was first introduced in France and England around 1600 for ornamental purposes (parks, botanical gardens), from where it spread throughout the continent and some parts of Asia [5]. Nowadays, black locust appears in many European countries, such as Germany, Hungary, Czech Republic, Poland, Slovakia, Austria and Romania [6,7], being added for forestry purposes. *R. pseudoacacia* has been studied because of its economic value rather than its ecology [8,9]. In Romania, it was introduced around 1750 and is considered an important woody species, imposing itself as a forest tree, even though black locust has multiple other uses and exploitation [10], with extensive plantations that cover an area of more than 2,306,000 ha [11].

As a species with rapid growth, *R. pseudoacacia* is also distinguished by the ease of its cultivation and the value of its wood, as it produces durable and rot-resistant wood [12]. Due to the fast vegetative development (production of 14 tons of dried subs/year/ha), it has potential for bio-oil production and fuel ethanol derived from biomass [13–16]. Black

locust's rusticity and ecological plasticity (it is the most cultivated exotic species in Europe) are other factors that have determined its spread and wide use. Even more, as part of the Fabaceae family, *R. pseudoacacia* is a nitrogen-fixing species [7], often used on abandoned fields and dry slopes [17]; it also grows on acid, dry and infertile sandy soils, and is also drought-tolerant [18]. Thus, the species is widely planted for revegetation and to control soil erosion. It is also considered a pioneer tree species due to its fast growth, resistance to pollution and strong capacity for improving soil nitrogen content and nutrient availability, the available phosphorus pool and organic carbon sequestration, as well as enhancing erodible sandy soil chemical and microbiological properties [19–22].

Nowadays, *Robinia* is considered as a melliferous species, with the honey collected being highly appreciated [23]; in Romania, there are almost 30 ha of qualified seed orchards, as well as other areas with provenance and melliferous (honey producing) orchards [10]. Other values to be mentioned for black locust are derived from processed products (e.g., wine from black-locust honey), dense wood that can be used for multiple purposes: habitat for insects, birds, fungi and birds and to increase carbon footprint [24]. Some areas lack highly productive native species with wood or growth characteristics suitable for forestry plantation; thus, there is a need to focus largely on exotic species, such as black locust, as it can be easily established on certain sites. Even though this is not necessarily the case for Romania, the species is considered valuable due to its wider physiological adaptability in terms of site conditions, especially on slopes, exploitations and sandy or erodible soils. In Romania, the area occupied by black locust has increased continuously [25,26]. Depending on the favorable ecological conditions, black locust could contribute in the same way as other forest species or by supplementing them, ensuring the multiple functions of the forest at local, zonal, regional, national or global levels [27–34]. The quality of the seeds is the first condition for the afforestation to be successful and for the resulting forests to fulfil their numerous functions; i.e., production, protection, ecological, cultural, educational, etc. [27,35,36]

R. pseudoacacia is considered to be a fast-growing species, as mentioned: in the first season (in the case of sprouts), it can reach heights of 6 m and get to heights of 30 m at the age of 30–35 years, with basal diameters of 30 cm at 15 years, depending on the type of regeneration and site conditions. Several black locust populations cultivated in Romania achieve average increases of 15–17 m^3/year/ha and reach the age of absolute exploitability at 30 years [27,37]. *R. pseudoacacia* reproduces sexually and asexually [17]. The first flowering occurs at the age of 5–6 years old, whereas the fruit is a pod that matures in autumn. A pod contains 4–10 dark brown seeds without endosperm, which are 4–6 mm in length. Every 1 or 2 years, it produces a high quantity of seeds [38]. Black locust seeds can be stored for more than 10 years at a temperature of 0–5 °C [39].

Normally, seed propagation is the easiest and most advantageous method of growing plants, but without previous treatments, black locust seed germination may be low due to the physiological dormancy induced by the impermeable tegument covering the seeds [40,41]. Seed propagation can be an easy and advantageous method of growing, but without previous treatments, seed germination may be low due to the physiological dormancy induced by the impermeable tegument [42], such as within *Robinia* species. The function of the seed coat has multiple benefits, such as protecting the embryo and endosperm from desiccation, mechanical injury, unfavourable temperatures and attacks by bacteria, fungi and insects, but breaking physical dormancy in some forest seeds is a challenge if one is to obtain homogeneous germination for larger seed samples. Both embryo and coat dormancies are components of physiological dormancy, and their interaction determine the degree of "whole-seed" physiological dormancy. The transition from dormancy to germination is a critical control point, leading to the initiation of vegetative growth.

Regarding the germination capacity, various chemical and mechanical treatments are used in forestry to improve the germination rate. Black locust seeds require pre-treatment before sowing in order to break exogenous dormancy, due to the structure of the seed coat, which is impermeable to water and gases [38]. Moreover, the imbibition and course of

processes that are essential for germination are inhibited in the dormancy stage [33,43]. The impermeability of the seed coat, understood as physical dormancy, is a very important ecological mechanism for species to ensure that germination occurs only in favorable conditions for seedling growth [44,45]. However, this phenomenon is undesirable when seeds are intended for commercial use and forest practice [46]. Thus, to break the dormancy of seeds, the seed coat must be damaged. Under natural conditions, the coat of black locust seeds can be damaged by either low (frosts) or high temperatures (fires) or by the activity of the soil microflora [47]. For research and practical purposes, three methods are used: mechanical (scarification), thermal and chemical [2,48].

The aim of the present study was to determine the efficiency of two treatments based on sulphuric acid at different concentrations and thermal shock treatment upon seeds to increase the germination rate of black locust seeds as well as to analyse the effect of the treatments and influence of the origin provenances on the development of seedlings in their first stage after emergence.

2. Materials and Methods

2.1. Biological Material

The seeds were collected from mature plus trees from eight Romanian provenances (Figure 1): (1) Galați, (2) Iași, (3) Botoșani, (4) Bihor, (5) Râmnicu Vâlcea, (6) Satu-Mare, (7) Bistrița-Năsăud and (8) Arad, corresponding to the Romanian Gene Reserved Forests and Seed Stands included in National Catalogue of Forest Genetic Resources and Forest Reproductive Materials [49].

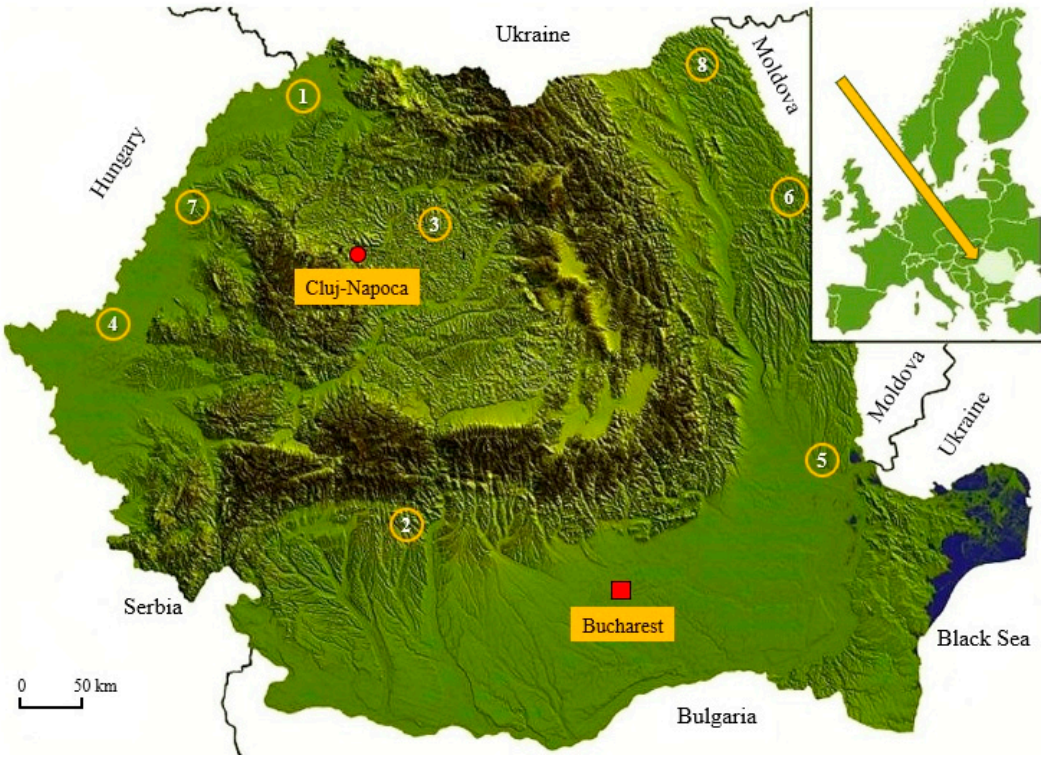


Figure 1. The Romanian provenances of *R. pseudoacacia* seeds.

To characterise the climatic heterogeneity of the different collection sites, mean annual temperature and mean precipitation were considered (Table 1).

Seeds were collected in the autumn of 2020. After harvesting, seeds were labeled for each provenance and deposited in paper bags in a refrigerator (at approximately +4 °C) until further analysis.

Table 1. Location and climatic characteristics of the eight *R. pseudoacacia* seeds provenances in Romania.

Provenance-Population	County	Administrative Location [1]	Latitude/ Longitude	Average Yearly Temperature (°C)	Average Annual Precipitation (mm)	Altitude (m asl)
Foieni	Satu Mare	OS Carei, UPIII, u.a.57L, 58A	47°42′ N/22°24′ E	10.9	619.2	130–130
Budești	Vâlcea	RNP Romsilva, OS Stoiceni UPII, u.a.5L, 5M	45°03′ N/24°26′ E	8.2	813.1	300–350
Pădurea Cetății	Bistrița Năsăud	RNP Romsilva, OSE Lechința UP.7, u.a.80C	47°00′ N/24°20′ E	6.3	802.9	380–400
Moneasa	Arad	Private Orchard Bărzani (Ruben Budău)	46°19′ N/21°40′ E	11.8	575.7	110–150
Drăgănești	Galați	RNP Romsilva, OS Tecuci UP.VI, u.a.49B	45°46′ N/27°30′ E	11.6	480.1	45–45
Borșa	Iași	RNP Romsilva, OS Iași UP. III, u.a.105E	47°25′ N/27°20′ E	10.5	540.9	60–160
Curtuișeni	Bihor	RNP Romsilva, OS Săcuieni UP. IV, u.a.45C	47°32′ N/22°09′ E	10.7	617.6	140–140
Liveni	Botoșani	RNP Romsilva, OS Darabani UP. III, u.a.26D	48°02′ N/27°01′ E	10.1	526.6	200–200

[1] Population description according to the National Catalogue of Forest Reproductive Materials, Bucharest (2012).

Moreover, since water stress is known to induce both morphological and anatomical changes [50–52], seedlings were also studied [53]. The seedlings obtained from treated seeds were further analysed by being kept in the laboratory for three months, then taken outside in natural conditions; at the end of six months, morphological traits were measured: height, stem diameter and number of branches.

2.2. Experimental Procedures Performed in Order to Investigate the Germination Rate

Seed analysis began in March 2021, following the established protocol and steps for the analysis of germination and seedling growth (Figure 2). Before starting the treatments, several measurements of the main phenotypic elements were performed for the seeds: height, diameter and weight. Two treatments were undertaken, each one with three concentrations, at three different temperatures. Four replicates, with 50 seeds per replicate, were used for each combination of population and treatment method. The first treatment involved water-soaked seeds and heat treatment (noted as thermal treatment), as follows: 1. seeds were soaked for 24 h in water at a temperature of 18 °C and then seeded and watered, while kept at room temperature; 2. seeds were stored at an air temperature of 45 °C for two hours (Memmet UF110 oven) and thereafter stored in a refrigerator at −20 °C for two hours and then seeded and watered; 3. seeds were stored at 60 °C for two hours, stored at −20 °C for a further two hours and then seeded and watered. The second treatment was based on sulphuric acid solution at different concentrations (50%, 70% and 90%), within which seeds were soaked for 20 min, at room temperature and light, and then seeded and watered.

For all treatments, watering was performed twice a week with tap water, for a total of four weeks of investigation; all replicates were kept at laboratory temperature (18 °C) and light. To calculate the germination rate, observations were made on days 4, 7, 10, 14, 21 and 28 for all treatments.

Figure 2. Stages of analysis of seed morphological characteristics, seed germination and seedling growth.

2.3. Statistical Analyses

The registered data of the seeds and seedlings were processed as the mean of the traits and standard error of the mean (SEM). An analysis of variance (ANOVA) was applied to the analysed traits, and if the null hypothesis was rejected, a post hoc test was used for the analysis of differences. Among the general class of multiple comparisons procedures, the most suitable for our data and sets of means comparison was considered to be Duncan's Multiple Range Test (Duncan's MRT, $p < 0.05$) [54]. The data were subjected to multivariate statistical analysis, namely principal component analysis (PCA). A multivariate principal components analysis graph for the eight provenances of *R. pseudoacacia* was created using Past software [55]. This software was used also for the construction of a dendrogram, as Euclidean distances among provenances.

3. Results

The treatments investigated to promote black locust germination—respectively, heat treatment and a chemical treatment using diluted sulphuric acid—had different results. Morphological trsaits of the seeds were measured and are presented in Table 2, indicating that their origin was relevant. The longest seeds were found from Bihor provenance, with distinct significative differences, while the shortest seeds were those from Bistrița Năsăud and Galați. In regard to the diameter of block locust seeds, the best value was recorded for Satu-Mare provenance. On the opposite side, the smallest diameter was obtained for seeds from Galați and Botoșani. The heaviest seeds were obtained from plus trees from Satu-Mare and Iași, followed by Bihor seeds.

It is worthwhile to note that the longest seeds were not the same as those that had the largest diameter, nor the heaviest seeds. The only provenance that had high values, meaning that the investigated traits were statistically superior, was Satu-Mare (Table 2).

Traits that have a CV% less than 10% are considered to have a low variability; in this regard, the most uniform length for the seeds was noted within Bihor provenance and the highest value for CV% was 16.9 for Bistrița N., which means the variability was medium for these seeds. Even more, CV% values between 20–30% can illustrate a large variability, and over 30% shows a very high variability [56]. It is to be further analysed if these results

correlate with the germination rate after the proposed treatments, so that conclusions can be made with regard to the interaction of seed traits and the capacity of germination. Data are presented in tables that summarise both treatments with all replicates, whereas the results are noted separately and as means, with the significance of differences (Tables 3–6).

Table 2. The main morphological traits of the black locust seeds, as mean * and standard error of the mean (SEM).

Provenance	Seeds Length (mm)		Seeds Width (mm)		Seeds Weight (g)	
	Mean ± SEM	CV%	Mean ± SEM	CV%	Mean ± SEM	CV%
Satu-Mare	3.13 ^{ab} ± 0.27	15.0	1.14 ^{a} ± 0.04	6.1	0.031 ^{a} ± 0.002	10.2
Vâlcea	3.00 ^{ab} ± 0.06	3.2	0.63 ^{cd} ± 0.05	14.6	0.022 ^{bc} ± 0.001	16.0
Bistrița N.	2.28 ^{c} ± 0.22	16.9	0.56 ^{cd} ± 0.19	5.7	0.017 ^{d} ± 0.001	4.5
Arad	2.77 ^{bc} ± 0.15	9.4	0.72 ^{bc} ± 0.10	22.9	0.024 ^{b} ± 0.001	7.7
Galați	2.36 ^{c} ± 0.10	7.3	0.40 ^{d} ± 0.02	8.3	0.019 ^{cd} ± 0.000	3.1
Iași	2.78 ^{bc} ± 0.13	8.4	0.89 ^{b} ± 0.10	19.2	0.031 ^{a} ± 0.000	1.1
Bihor	3.25 ^{a} ± 0.03	1.4	0.61 ^{cd} ± 0.09	24.1	0.028 ^{a} ± 0.002	11.3
Botoșani	2.71 ^{bc} ± 0.15	9.6	0.43 ^{d} ± 0.06	23.7	0.023 ^{bc} ± 0.000	11.5

* The means on the column followed by different letters are significantly different according to Duncan's MRT test ($p < 0.05$).

Table 3. Black locust seed germination (%) depending on the treatments applied.

Provenance	Sulphuric Acid Treatment/Concentration			Mean Provenance **
	50%	70%	90%	
Satu-Mare	5.5 ^{gh} *	15.8 $^{e–g}$	40.0 ^{ab}	20.4 ^{CD}
Vâlcea	45.3 ^{a}	21.5 $^{d–f}$	30.0 $^{b–d}$	32.3 ^{A}
Bistrița N.	30.5 $^{b–d}$	25.0 $^{c–e}$	34.8 $^{a–c}$	30.1 ^{AB}
Arad	39.8 ^{ab}	10.3 $^{f–h}$	25.5 $^{c–e}$	25.2 $^{A–C}$
Galați	39.8 ^{ab}	16.0 $^{e–g}$	20.5 $^{d–f}$	25.4 $^{A–C}$
Iași	40.8 ^{ab}	16.3 $^{e–g}$	26.8 $^{c–e}$	27.9 $^{A–C}$
Bihor	0.0 ^{h}	34.8 $^{a–c}$	0.0 ^{h}	11.6 ^{D}
Botoșani	45.0 ^{a}	4.8 ^{gh}	20.5 $^{d–f}$	23.4 ^{CD}
Mean treatment ***	30.8 ^{Z}	18.0 ^{Y}	24.7 ^{X}	-
Provenance	**Thermal Treatment/Temperatures**			**Mean Provenance**
	18 °C	**45 °C, −20 °C**	**60 °C, −20 °C**	
Satu-Mare	20.5 ^{ij}	49.8 $^{b–d}$	10.3 ^{k}	26.8 ^{EF}
Vâlcea	30.0 $^{g–j}$	37.0 $^{d–h}$	43.8 $^{b–f}$	36.9 ^{CD}
Bistrița N.	40.3 $^{c–g}$	25.0 $^{h–j}$	31.0 $^{f–j}$	32.1 ^{DE}
Arad	25.5 $^{h–j}$	25.5 $^{h–j}$	20.0 $^{i–j}$	23.7 ^{F}
Galați	70.8 ^{a}	20.8 ^{ij}	49.8 $^{b–d}$	47.1 ^{B}
Iași	51.8 ^{bc}	46.8 $^{b–e}$	34.5 $^{e–i}$	44.3 ^{BC}
Bihor	55.3 ^{b}	75.0 ^{a}	74.3 ^{a}	68.2 ^{A}
Botoșani	30.5 $^{f–j}$	50.0 $^{b–d}$	55.0 ^{b}	45.2 ^{BC}
Mean treatment	40.6 ^{Z}	41.2 ^{Z}	39.8 ^{Z}	-

* The means on the column inside the table followed by different small letters are significantly different according to Duncan's MRT test ($p < 0.05$). ** The means on the last column reflect the influence of the provenances, regardless of the treatment applied. The means followed by different capital letters are significantly different according to Duncan's MRT test ($p < 0.05$). *** The means on the last row reflect the influence of the treatments, regardless of the provenances. The means followed by different capital letters are significantly different according to Duncan's MRT test ($p < 0.05$).

Regarding seed germination, the obtained results indicated that the highest germination rate for black locust occurred in the water and temperature variation treatment on seeds from Bihor provenance (68.2%) (Table 3); interestingly, the same location had the smallest values of germination when seeds were treated with sulphuric acid (11.6%). Thus,

it can be said that the provenance of seeds and the different treatments had a significant effect on the dynamics of germination.

Table 4. Seedling's height (cm) depending on the treatments applied to black locust seeds.

Provenance	Sulphuric Acid Treatment/Concentration			Mean Provenance **
	50%	70%	90%	
Satu-Mare	24.5 $^{f–j}$ *	28.5 $^{e–h}$	31.5 $^{c–f}$	28.2 ^{BC}
Vâlcea	10.8 ^{l}	8.3 ^{l}	9.3 ^{l}	9.4 ^{E}
Bistrița N.	20.3 $^{h–k}$	19.5 $^{i–k}$	21.5 $^{g–k}$	20.4 ^{D}
Arad	45.0 ^{a}	37.5 $^{a–d}$	40.5 ^{ab}	41.0 ^{A}
Galați	30.0 $^{d–g}$	24.0 $^{f–k}$	27.5 $^{e–i}$	27.2 ^{C}
Iași	39.3 $^{a–c}$	29.8 $^{d–g}$	33.8 $^{b–e}$	34.3 ^{B}
Bihor	0.0 ^{m}	31.8 $^{c–f}$	0.0 ^{m}	10.6 ^{E}
Botoșani	22.0 $^{g–k}$	15.5 $^{k–l}$	18.8 ^{jk}	18.8 ^{D}
Mean treatment ***	24.0 ^{x}	24.3 ^{x}	22.8 ^{x}	-
Provenance	**Thermal Treatment/Temperatures**			**Mean Provenance**
	18 °C	**45 °C, −20 °C**	**60 °C, −20 °C**	
Satu-Mare	24.0 $^{h–l}$	31.0 $^{e–g}$	18.8 ^{kl}	24.6 ^{C}
Vâlcea	8.0 ^{m}	8.6 ^{m}	9.5 ^{m}	8.7 ^{F}
Bistrița N.	21.0 $^{j–l}$	17.6 ^{l}	20.0 $^{k–l}$	19.5 ^{E}
Arad	45.0 ^{a}	42.5 ^{ab}	38.5 ^{bc}	42.0 ^{A}
Galați	26.9 $^{f–j}$	18.7 ^{kl}	25.3 $^{g–k}$	23.6 ^{CD}
Iași	38.0 $^{b–d}$	36.0 $^{c–e}$	30.8 $^{e–g}$	34.9 ^{A}
Bihor	28.5 $^{f–i}$	30.0 $^{e–h}$	32.1 $^{d–f}$	30.2 ^{B}
Botoșani	17.5 ^{l}	22.0 $^{i–l}$	23.8 $^{h–l}$	21.1 ^{DE}
Mean treatment	26.1 ^{X}	25.8 ^{X}	24.8 ^{X}	-

* The means on the column inside the table followed by different small letters are significantly different according to Duncan's MRT test ($p < 0.05$). ** The means on the last column reflect the influence of the provenances, regardless of the treatment applied. The means followed by different capital letters are significantly different according to Duncan's MRT test ($p < 0.05$). *** The means on the last row reflect the influence of the treatments, regardless of the provenances. The means followed by different capital letters are significantly different according to Duncan's MRT test ($p < 0.05$).

Even more, the germination rate was significantly higher for the water treatment compared with the sulphuric acid treatment (Table 3). The differences were consistent among provenances, but the strongest impact was found for the applied treatment. The highest percentage of seed germination was obtained for water and 45 °C temperature treatment (41.2%), compared with the other values obtained, which ranged between 18.0% and 30.8% for different concentrations of sulphuric acid treatment.

The results obtained were directly influenced by the concentration of acid (Table 3); in the investigation, the exposure of seeds to 50% sulphuric acid for 20 min resulted in the highest mean for germination for the relevant treatment (30.8% compared with 18% and 24.7%).

Overall, the seeds treated within the thermal variations treatment (45 °C, −20 °C) had the highest germination rate (41.2%). Even so, the water and temperature treatment applied better stimulated the germination of black locust seeds (ranging between 39.8–41.2%) (Table 3).

Depending on the concentration, the doses of 50% and 90% of sulphuric acid determined the continuous increase of the percentage of germinated seeds, after 4, 7, 10 and 14 days from the treatment, after which there was a decrease in the proportion of germinated black locust seeds until the 21 day interval (Figure 3). The regression equation for the 70% concentration did not ensure the same upward trend of the regression line. However, at a concentration of 70%, the coefficient of determination showed the largest contribution of the independent variable (the time interval in which the germination was analysed) of the total variance (37.3%). In addition, it was noted that the correlation coefficient had the highest value (0.611).

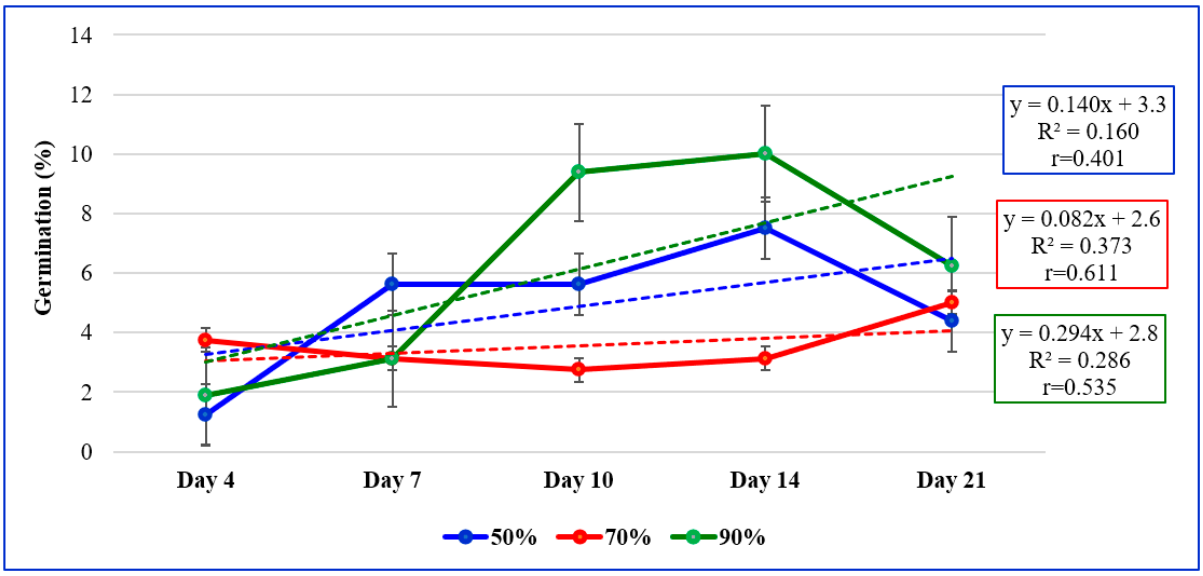


Figure 3. Black locust seed germination (%) evolution in days, depending on the treatments applied with three concentrations of sulphuric acid.

Table 5. Seedling diameter (cm) depending on the treatments applied to black locust seeds.

Provenance	Sulphuric Acid Treatment/Concentration			Mean Provenance **
	50%	70%	90%	
Satu-Mare	3.2 $^{c–g}$ *	4.3 $^{a–d}$	5.2 ^{a}	4.2 ^{A}
Vâlcea	5.0 ^{a}	3.7 $^{b–f}$	4.4 $^{a–c}$	4.3 ^{A}
Bistrița N.	2.2 ^{g}	2.5 ^{fg}	3.1 $^{d–g}$	2.6 ^{B}
Arad	5.3 ^{a}	4.0 $^{a–e}$	4.8 ^{ab}	4.7 ^{A}
Galați	3.1 $^{d–g}$	2.2 ^{g}	3.0 $^{e–g}$	2.7 ^{B}
Iași	3.5 $^{c–g}$	2.3 ^{g}	2.6 ^{fg}	2.8 ^{A}
Bihor	0.0 ^{h}	3.3 $^{c–g}$	0.0 ^{h}	1.1 ^{C}
Botoșani	3.1 $^{c–g}$	2.3 ^{g}	2.5 ^{fg}	2.6 ^{B}
Mean treatment ***	3.1 ^{X}	3.1 ^{X}	3.2 ^{X}	-
Provenance	**Thermal Treatment/Temperatures**			**Mean Provenance**
	18 °C	**45 °C, −20 °C**	**60 °C, −20 °C**	
Satu-Mare	4.2 $^{a–e}$	5.3 ^{a}	3.7 $^{b–g}$	4.4 ^{AB}
Vâlcea	3.1 $^{c–g}$	4.0 $^{a–e}$	4.3 $^{a–d}$	3.8 ^{BC}
Bistrița N.	3.3 $^{c–g}$	2.8 $^{e–g}$	3.1 $^{c–g}$	3.0 ^{CD}
Arad	5.3 ^{a}	4.7 ^{ab}	4.5 $^{a–c}$	4.8 ^{A}
Galați	2.8 $^{e–g}$	2.3 ^{g}	3.0 $^{d–g}$	2.7 ^{D}
Iași	3.8 $^{b–f}$	3.0 $^{d–g}$	2.9 $^{e–g}$	3.2 ^{CD}
Bihor	2.9 $^{d–g}$	3.0 $^{d–g}$	3.5 $^{b–g}$	3.1 ^{CD}
Botoșani	2.5 $^{f–g}$	3.4 $^{b–g}$	3.5 $^{b–g}$	3.1 ^{CD}
Mean treatment	3.5 ^{X}	3.5 ^{X}	3.7 ^{X}	-

* The means on the column inside the table followed by different small letters are significantly different according to Duncan's MRT test ($p < 0.05$). ** The means on the last column reflect the influence of the provenances, regardless of the treatment applied. The means followed by different capital letters are significantly different according to Duncan's MRT test ($p < 0.05$). *** The means on the last row reflect the influence of the treatments, regardless of the provenances. The means followed by different capital letters are significantly different according to Duncan's MRT test ($p < 0.05$).

The three types of thermal variation within the treatments applied to the seeds provided relatively close equations and regression lines (Figure 4). The coefficients of determination had quite low values, whereas the lowest value of the correlation coefficient was registered in the case of the treatment in which the high temperature (+60 °C) was alternated with the low temperature (−20 °C).

Table 6. Seedlings' number of branches per stem depending on the treatments applied to black locust seeds.

Provenance	Sulphuric Acid Treatment/Concentration			Mean Provenance **
	50%	70%	90%	
Satu-Mare	7 $^{d–f}$ *	7 $^{d–f}$	11.9 ^{a}	8.6 ^{AB}
Vâlcea	9 $^{b–d}$	7 $^{d–f}$	7 $^{d–f}$	7.7 ^{BC}
Bistrița N.	8 $^{c–e}$	6 ^{ef}	10 $^{a–c}$	8.0 ^{AB}
Arad	11 ^{ab}	9.1 ^{bd}	8 $^{c–e}$	9.4 ^{A}
Galați	8 $^{c–e}$	5 ^{f}	6 ^{ef}	6.3 ^{CD}
Iași	10 $^{a–c}$	7 $^{d–f}$	8 $^{c–e}$	8.3 ^{AB}
Bihor	0 ^{g}	11 ^{ab}	0 ^{g}	3.7 ^{E}
Botoșani	7 $^{d–f}$	4.9 ^{f}	6 ^{ef}	6.0 ^{D}
Mean treatment ***	7.5 ^{X}	7.1 ^{X}	7.1 ^{X}	-
Provenance	**Thermal Treatment/Temperatures**			**Mean Provenance**
	18 °C	**45 °C, −20 °C**	**60 °C, −20 °C**	
Satu-Mare	9 $^{d–e}$	12 ^{a}	7 $^{d–g}$	9.3 ^{AB}
Vâlcea	6 ^{fg}	7 $^{d–g}$	7 $^{d–g}$	6.7 ^{CD}
Bistrița N.	10 $^{a–c}$	6 ^{fg}	7 $^{d–g}$	7.7 ^{BC}
Arad	9.3 $^{b–d}$	9 $^{d–e}$	7 $^{d–g}$	8.4 ^{B}
Galați	6.3 $^{e–g}$	5 ^{g}	6 ^{fg}	5.8 ^{D}
Iași	9.8 $^{a–d}$	8.3 $^{c–f}$	6.3 $^{e–g}$	8.1 ^{BC}
Bihor	10.0 $^{a–c}$	11.8 ^{ab}	10.8 $^{a–c}$	10.8 ^{A}
Botoșani	7.3 $^{d–g}$	8 $^{c–f}$	8.3 $^{c–f}$	7.8 ^{BC}
Mean treatment	8.4 ^{X}	8.4 ^{X}	7.4 ^{Y}	-

* The means on the column inside the table followed by different small letters are significantly different according to Duncan's MRT test ($p < 0.05$). ** The means on the last column reflect the influence of the provenances, regardless of the treatment applied. The means followed by different capital letters are significantly different according to Duncan's MRT test ($p < 0.05$). *** The means on the last row reflect the influence of the treatments, regardless of the provenances. The means followed by different capital letters are significantly different according to Duncan's MRT test ($p < 0.05$).

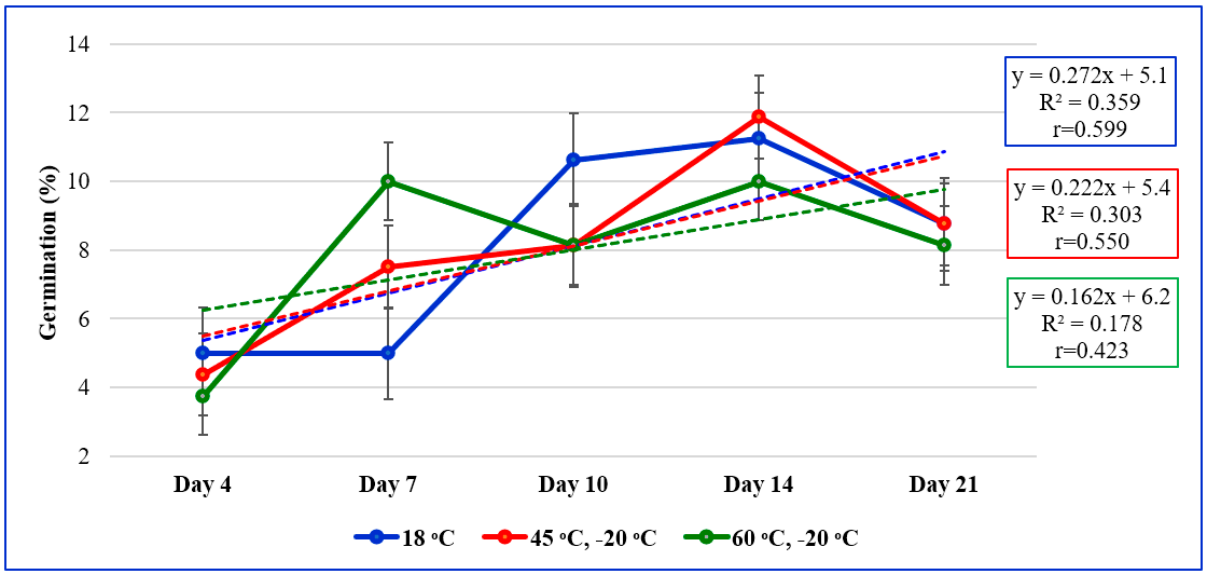


Figure 4. Black locust seed germination (%) evolution in days, depending on the thermal treatments applied with three different levels.

Regarding the seedlings' height, it was noted that Arad provenance seeds had higher values in both treatments (41.0 cm for sulphuric acid treatment and 42.0 cm for thermal treatment). On the other hand, the data obtained for Vâlcea seeds had the smallest values (9.4 cm

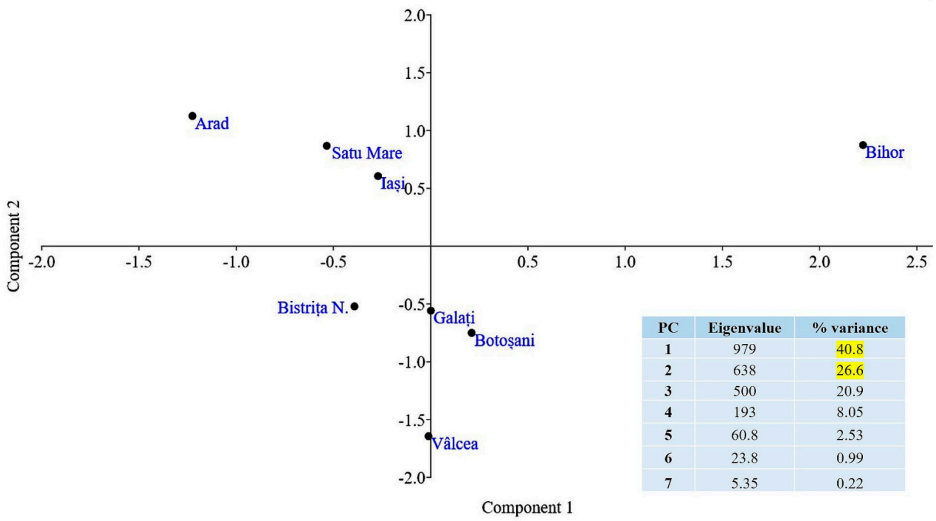
and 8.7 cm, respectively), with significant differences from the rest of the provenances (Table 4). The smaller concentrations of sulphuric acid tested (50 and 70%) performed better in the current investigation, even though the differences were not statistically assured.

The investigation of the diameter of seedlings confirmed that seeds from Arad provenance are valuable, meaning that the highest values were statistically assured (4.7 cm for acid treatment and 4.8 cm for temperature treatment) (Table 5). Even more, the water imbibition and exposure to heat/low temperature positively influenced the growth of the seedlings after thermal shock, compared with sulphuric acid, both for black locust seedlings' height and diameter.

With reference to the number of branches, it was noted that seeds from Bihor provenance showed different results. Seeds treated with sulphuric acid had the smallest number of ramifications (3.7), while in the water and temperature treatment, the same provenance gave the highest number of branches (10.8) (Table 6).

Different populations of *R. pseudoacacia* show significant differences in terms of seed characteristics and germination capacity, but also variability in terms of the growth and development of seedlings, potentially increasing the ability of this species to adapt to different environmental conditions.

Because the data sets obtained from the combinations of provenances and treatments were large, referring to both seeds and seedlings, it was intended to reduce their size with the help of PCA but maintain all information. The principal component analysis (PCA) illustrated in Figure 5 provides a presentation of the "variability" of the variables, while retaining all the elements analysed in the current study. By identifying new variables as linear functions of those in the original data set, it was found that the first component of the PCA, respectively PC1, accounted for 40.8%, while the second component (PC2) accounted for 26.6% of the total variation observed. The values of these new variables, which successively maximize the variance, and even more are uncorrelated with each other, allowed the reduction of data sets and the summarisation of the eigenvalue problems in a descriptive rather than inferential way. Of the eight Romanian provenances, Satu-Mare and Iași form a relatively homogeneous group, but they are in opposition with Galați and Botoșani, which form another homogeneous group. The most distant provenance is Bihor, which is in a negative correlation with Bistrița Năsăud.



PC	Eigenvalue	% variance
1	979	40.8
2	638	26.6
3	500	20.9
4	193	8.05
5	60.8	2.53
6	23.8	0.99
7	5.35	0.22

Figure 5. The hierarchical dendrogram of cluster analysis–paired group UPGMA (Unweighted Pair Group Method with Arithmetic Mean)–similarity index (Euclidean), based on the analysed parameters of the *R. pseudoacacia* provenances.

Furthermore, the obtained dendrogram (Figure 6) shows the clusters of the provenances according to the grouping of the observations made and the levels of similarity. The tree diagram confirms the results presented above, both by the pattern by which the

clusters were formed and also by the similarity or distance levels of the clusters that contain the analysed *R. pseudoacacia* provenances.

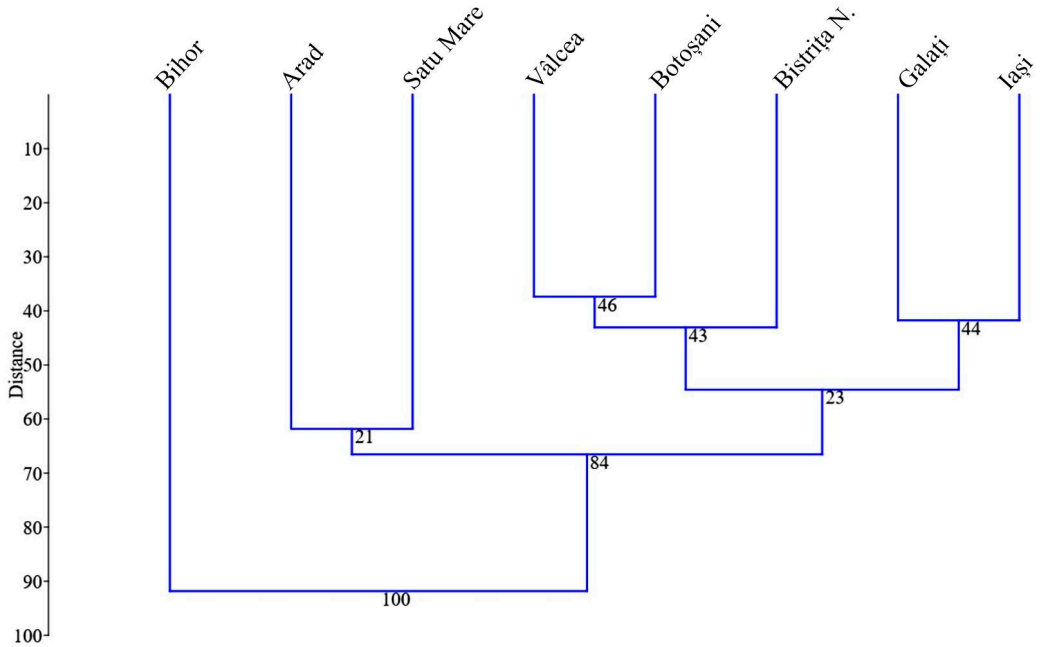


Figure 6. The hierarchical dendrogram of cluster analysis based on the analysed parameters of the *R. pseudoacacia* provenances.

As stated in the discussion about PCA analysis, the distinct cluster of Bihor provenance is obvious. Another cluster consists of Arad and Satu-Mare, separated by a third cluster, the last with two distinct subclusters. Of these subclusters, the one located at the right end of the dendrogram consists of two provenances with certain geographical proximity (Galați and Iași). The other subcluster, which occurs at a close similarity level, contains three provenances—two grouped together at the same level of similarity or distance (Vâlcea and Botoșani) and a separate one (Bistrița Năsăud).

4. Discussion

The management of genetic resources of forest species and their efficient conservation suffer from variations in traits and seed germination [57]. Morphological traits noted for black locust seeds indicated that the origin was relevant for the current investigation, with the data being quite variable. The differences among investigated populations were statistically assured, and the germination was surely influenced by the ecological parameters in the area of provenance, along with the applied treatment.

The environmental heterogeneity of the black locust seed origin within the Romanian provenances may finally result in different germination performances. Indeed, variability in the germination responses of seeds from different collection sites is well documented even in other Fabaceae species [58,59]. According to the specialty literature, larger seeds tend to have a higher germination rate, mainly because it is assumed they contain more resources to support such an intense biological process, which needs a great deal of energy [60] and also leads to healthier seedlings [61]. In the current investigation, Bihor had the largest seeds and also promoted a good germination within the thermal treatment. It is interesting to further analyse the fact that Satu-Mare seeds, which had significant higher values for seed traits, were not found among the provenances with a good germination within all treatments, nor for seedlings with a significant height and diameter. The longest seeds were found from Bihor provenance, with distinct significative differences, which also had the best germination rate for the thermal treatment; interestingly, however, seeds from the

same origin also had the poorest germination after being treated with sulphuric acid at the three concentrations tested.

For the germination assay, high germination rates were obtained by immersing seeds in boiled water at temperatures of 60–80 °C, with a soaking time between 20 min to 72 h and several heating water cycles/cooling [62,63]. It can be concluded that the results can be influenced by the treatment that should destroy the seeds' tegument, on which the physiological parameters can interact. Thus, seeds collected in sites characterised by different environmental parameters display a noteworthy difference in the germination dynamics [53], and this aspect should always be considered when procuring biological material and correlated with the final goal.

There are several studies that have shown that the application of sulphuric acid can be more effective compared with other treatments for *R. pseudoacacia* seed germination [48,60,64]. Black locust seeds have a hard and waterproof tegument [65], meaning that scarification is usually applied [61]. Nevertheless [7], seeds soaked for 24 h stimulated a final value of 8% as a germination percentage. By heating the seeds for two hours at 45 °C and 60 °C, followed by two hours at 20 °C as a hot–cold variation, germination reached 23% and 69%, respectively. Thus, the variation among treatments is real, and the best method is still under investigation. Furthermore, in the current investigation, treatments with sulphuric acid were less efficient (the values ranged between 18–30.8%) than the thermic shock (values between 39.8 and 41.2%). Even more, it was observed [7] that the best results on germination capacity were obtained by mechanical scarification [66]. Furthermore, the experiment showed that germination capacity increased with the best results after extreme hot–cold thermal variation (60 °C, −20 °C). It is assumed that after thermal shock, micro-cracks appear in the tegument, meaning that water enters rapidly and light stimuli facilitate the growth processes.

The treatments applied to black locust seeds with both sulphuric acid and thermal differences influenced the germination during the period in which the observations were made. As a whole, the current results confirmed the phenotypic plasticity of black locust as a response to variable water availability and provided evidence for the potential high germination capacity when thermal shock is properly managed. It is important to note the practical results obtained and the resulting grouping of the data, using principal component analysis, for black locust seed germination and the hierarchical dendrogram of cluster analysis; in this context, Bihor provenance stands out, followed by Arad and Satu-Mare, which were at the same level of similarity, as seen in the tree dendogram. It will be interesting to further investigate the possibility to test the seedlings in an experimental field and conduct different studies of similarities (differences) upon their growth.

The geographical area potentially occupied or invaded by *R. pseudoacacia* is expected to increase significantly under the future scenario of global warming [67], despite the germination difficulties. Hence, it is highly recommended to integrate bio-ecological information and to study the species' behaviour and its adaptive capacity [68] under varying environmental factors, with the aim of planning its management and control [69,70]. Conversely, the species tolerates drastic variations of soil water availability in Central Europe and is thus becoming an important tree species to be cultivated on dry, even marginal lands [71]. Nevertheless, the black locust genus is not native to Romania, and some even consider it invasive, but its uses can overcome this inconvenience when proper techniques are applied and the cultivation is made rationally.

5. Conclusions

Germination assays performed in the current study revealed a noteworthy variability in seed germination responses across provenances. The different germination behaviour of *R. pseudoacacia* seeds of the examined Romanian populations may be related to the different environmental parameters of the collection sites. The thermal shock applied to black locust seeds can be concluded to be more efficient with regard to their germination compared with the sulphuric acid treatment. The exposure to heat/cold significantly improved

germination but also black locust seedlings' height, diameter and number of branches. As a whole, the current results confirmed that the phenotypic plasticity of black locust may be a response to variable ecological parameters and provided evidence for its potential high germination capacity with proper treatments.

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References

1. Rédei, K.; Keserű, Z.; Rásó, J. Early evaluation of micropropagated black locust (*Robinia pseudoacacia* L.) clones in Hungary. *For. Sci. Pract.* **2013**, *15*, 81–84. [CrossRef]
2. Jastrzębowski, S.; Ukalska, J.; Kantorowicz, W.; Klisz, M.; Wojda, T.; Sułkowska, M. Effects of thermal-time artificial scarification on the germination dynamics of black locust (*Robinia pseudoacacia* L.) seeds. *Eur. J. For. Res.* **2017**, *136*, 471–479. [CrossRef]
3. Huntley, B. European vegetation history: Palaeovegetation maps from pollen data-13 000 yr BP to present. *J. Quat. Sci.* **1990**, *5*, 103–122. [CrossRef]
4. Pepe, M.; Gratani, L.; Fabrini, G.; Varone, L. Seed germination traits of Ailanthus altissima, Phytolacca americana and *Robinia pseudoacacia* in response to different thermal and light requirements. *Plant Species Biol.* **2020**, *35*, 300–314. [CrossRef]
5. Keresztesi, B. Black Locust: The Tree of Agriculture. *Outlook Agric.* **1988**, *17*, 77–85. [CrossRef]
6. Vítková, M.; Müllerová, J.; Sádlo, J.; Pergl, J.; Pyšek, P. Black locust (*Robinia pseudoacacia*) beloved and despised: A story of an invasive tree in Central Europe. *For. Ecol. Manag.* **2017**, *384*, 287–302. [CrossRef] [PubMed]
7. Carl, C.; Lehmann, J.R.K.; Landgraf, D.; Pretzsch, H. *Robinia pseudoacacia* L. in Short Rotation Coppice: Seed and Stump Shoot Reproduction as well as UAS-based Spreading Analysis. *Forests* **2019**, *10*, 235. [CrossRef]
8. Lukasiewicz, M.; Kowalski, S.; Makarewicz, M. Antimicrobial an antioxidant activity of selected Polish herbhoneys. *LWT* **2015**, *64*, 547–553. [CrossRef]
9. Quinkenstein, A.; Jochheim, H. Assessing the carbon sequestration potential of poplar and black locust short rotation coppices on mine reclamation sites in Eastern Germany—Model development and application. *J. Environ. Manag.* **2016**, *168*, 53–66. [CrossRef] [PubMed]
10. Nicolescu, V.-N.; Hernea, C.; Bakti, B.; Keserű, Z.; Antal, B.; Rédei, K. Black locust (*Robinia pseudoacacia* L.) as a multi-purpose tree species in Hungary and Romania: A review. *J. For. Res.* **2018**, *29*, 1449–1463. [CrossRef]
11. Brus, R. Current Occurrence of Non-Native Tree Species in European Forest. In Proceedings of the Joint WG Meeting of COST Action 402 Non-Native Tree Species for European Forests Experiences, Risks and Opportunities (NNEXT), Lisbon, Portugal, 4 October 2016.
12. Tateno, R.; Tokuchi, N.; Yamanaka, N.; Du, S.; Otsuki, K.; Shimamura, T.; Xue, Z.; Wang, S.; Hou, Q. Comparison of litterfall production and leaf litter decomposition between an exotic black locust plantation and an indigenous oak forest near Yan'an on the Loess Plateau, China. *For. Ecol. Manag.* **2007**, *241*, 84–90. [CrossRef]
13. Grünewald, H.; Böhm, C.; Quinkenstein, A.; Grundmann, P.; Eberts, J.; Von Wühlisch, G. *Robinia pseudoacacia* L.: A Lesser Known Tree Species for Biomass Production. *BioEnergy Res.* **2009**, *2*, 123–133. [CrossRef]
14. Balat, M. Bio-Oil Production from Pyrolysis of Black Locust (*Robinia pseudoacacia*) Wood. *Energy Explor. Exploit.* **2010**, *28*, 173–186. [CrossRef]
15. González-García, S.; Gasol, C.M.; Moreira, M.T.; Gabarrell, X.; i Pons, J.R.; Feijoo, G. Environmental assessment of black locust (*Robinia pseudoacacia* L.)-based ethanol as potential transport fuel. *Int. J. Life Cycle Assess.* **2011**, *16*, 465–477. [CrossRef]
16. Mally, R.; Ward, S.F.; Trombik, J.; Buszko, J.; Medzihorský, V.; Liebhold, A.M. Non-native plant drives the spatial dynamics of its herbivores: The case of black locust (*Robinia pseudoacacia*) in Europe. *NeoBiota* **2021**, *69*, 155–175. [CrossRef]
17. Cierjacks, A.; Kowarik, I.; Joshi, J.; Hempel, S.; Ristow, M.; von der Lippe, M.; Weber, E. Biological Flora of the British Isles: *Robinia pseudoacacia* . *J. Ecol.* **2013**, *101*, 1623–1640. [CrossRef]

18. Wu, W.; Li, H.; Feng, H.; Si, B.; Chen, G.; Meng, T.; Li, Y.; Siddique, K.H. Precipitation dominates the transpiration of both the economic forest (*Malus pumila*) and ecological forest (*Robinia pseudoacacia*) on the Loess Plateau after about 15 years of water depletion in deep soil. *Agric. For. Meteorol.* **2020**, *297*, 108244. [CrossRef]
19. Ussiri, D.A.N.; Lal, R.; Jacinthe, P.A. Soil Properties and Carbon Sequestration of Afforested Pastures in Reclaimed Minesoils of Ohio. *Soil Sci. Soc. Am. J.* **2006**, *70*, 1797–1806. [CrossRef]
20. Qiu, L.; Zhang, X.; Cheng, J.; Yin, X. Effects of black locust (*Robinia pseudoacacia*) on soil properties in the loessial gully region of the Loess Plateau, China. *Plant Soil* **2010**, *332*, 207–217. [CrossRef]
21. Wang, B.; Liu, G.; Xue, S. Effect of black locust (*Robinia pseudoacacia*) on soil chemical and microbiological properties in the eroded hilly area of China's Loess Plateau. *Environ. Earth Sci.* **2011**, *65*, 597–607. [CrossRef]
22. Zhang, H.; Liu, Z.; Chen, H.; Tang, M. Symbiosis of Arbuscular Mycorrhizal Fungi and *Robinia pseudoacacia* L. Improves Root Tensile Strength and Soil Aggregate Stability. *PLoS ONE* **2016**, *11*, e0153378. [CrossRef] [PubMed]
23. Dalby, R. A honey of a tree: Black locust. *Am. Bee J.* **2004**, *144*, 382–384.
24. Spyroglou, G.; Fotelli, M.; Nanos, N.; Radoglou, K. Assessing Black Locust Biomass Accumulation in Restoration Plantations. *Forests* **2021**, *12*, 1477. [CrossRef]
25. Ciuvăț, A.L.; Abrudan, I.V.; Blujdea, V.; Marcu, C.; Dinu, C.; Enescu, C.; Nuță, I.S. Distribution and peculiarities of black locust in Romania. *Rev. Silvic. Cinegetică* **2013**, *32*, 76–85.
26. Sitzia, T.; Cierjacks, A.; de Rigo, D.; Caudullo, G. Robinia pseudoacacia in Europe: Distribution, habitat, usage and threats. In *European Atlas of Forest Tree Species*; San-Miguel-Ayanz, J., de Rigo, D., Caudullo, G., Houston Durrant, T., Mauri, A., Eds.; Publication Office of the European Union: Luxembourg, 2016; pp. 166–167.
27. Nicolescu, V.-N.; Rédei, K.; Mason, W.L.; Vor, T.; Pöetzelsberger, E.; Bastien, J.-C.; Brus, R.; Benčať, T.; Đodan, M.; Cvjetkovic, B.; et al. Ecology, growth and management of black locust (*Robinia pseudoacacia* L.), a non-native species integrated into European forests. *J. For. Res.* **2020**, *31*, 1081–1101. [CrossRef]
28. Matei, I.; Pacurar, I.; Roșca, S.; Bilașco, Ș.; Sestras, P.; Rusu, T.; Jude, E.T.; Tăut, F.D. Land Use Favourability Assessment Based on Soil Characteristics and Anthropic Pollution. Case Study Somesul Mic Valley Corridor, Romania. *Agronomy* **2020**, *10*, 1245. [CrossRef]
29. Curovic, M.; Spalevic, V.; Sestras, P.; Motta, R.; Dan, C.; Garbarino, M.; Vitali, A.; Urbinati, C. Structural and ecological characteristics of mixed broadleaved old-growth forest (Biogradska Gora-Montenegro). *Turk. J. Agric. For.* **2020**, *44*, 428–438. [CrossRef]
30. Kraszkiewicz, A. Productivity of Black Locust (*Robinia pseudoacacia* L.) Grown on a Varying Habitats in Southeastern Poland. *Forests* **2021**, *12*, 470. [CrossRef]
31. Deneau, K.A. The Effects of Black Locust (*Robinia pseudoacacia* L.) on Understory Vegetation and Soils in a Northern Hardwood Forest. Master's Thesis, Michigan Technological University, Houghton, MI, USA, 2013.
32. Annighöfer, P.; Mölder, I.; Zerbe, S.; Kawaletz, H.; Terwei, A.; Ammer, C. Biomass functions for the two alien tree species Prunus serotina Ehrh. and *Robinia pseudoacacia* L. in floodplain forests of Northern Italy. *Eur. J. For. Res.* **2012**, *131*, 1619–1635. [CrossRef]
33. Wei, X.; Liang, W. Multifactor relationships between stand structure and soil and water conservation functions of *Robinia pseudoacacia* L. in the Loess Region. *PLoS ONE* **2019**, *14*, e0219499. [CrossRef]
34. Allam, A.; Borsali, A.H.; Kefifa, A.; Zouidi, M.; DA Silva, A.M.F.; Rébufa, C. Impact of water erosion on the properties of forest soils. *Not. Sci. Biol.* **2021**, *13*, 10921. [CrossRef]
35. Sestras, P.; Bondrea, M.V.; Cetean, H.; Sălăgean, T.; Bilaşco, Ş.; Naș, S.; Spalevic, V.; Fountas, S.; Cîmpeanu, S.M. Ameliorative, Ecological and Landscape Roles of Făget Forest, Cluj-Napoca, Romania, and Possibilities of Avoiding Risks Based on GIS Landslide Susceptibility Map. *Not. Bot. Horti Agrobot. Cluj-Napoca* **2017**, *46*, 292–300. [CrossRef]
36. Hou, G.; Bi, H.; Wei, X.; Wang, N.; Cui, Y.; Zhao, D.; Ma, X.; Wang, S. Optimal configuration of stand structures in a low-efficiency *Robinia pseudoacacia* forest based on a comprehensive index of soil and water conservation ecological benefits. *Ecol. Indic.* **2020**, *114*, 106308. [CrossRef]
37. Ciuvat, A.L.; Abrudan, I.V.; Blujdea, V.; Dutca, I.; Nuta, I.S.; Edu, E. Biomass Equations and Carbon Content of Young Black Locust (*Robinia pseudoacacia* L.) Trees from Plantations and Coppices on Sandy Soils in South-Western Romanian Plain. *Not. Bot. Horti Agrobot. Cluj-Napoca* **2013**, *41*, 590–592. [CrossRef]
38. Olson, D.F.J.; Karrfalt, R.P. *The Woody Plant Seed Manual*; Robinia, L.L., Bonner, F., Karrfalt, R.P., Eds.; Agriculture Handbook; USDA Forest Service: Washington, DC, USA, 2008; Volume 727.
39. DeGomez, T.; Wagner, M.R. Culture and Use of Black Locust. *HortTechnology* **2001**, *11*, 279–288. [CrossRef]
40. Rédei, K.; Csiha, I.; Keserű, Z.; Gál, J. Influence of Regeneration Method on the Yield and Stem Quality of Black Locust (*Robinia pseudoacacia* L.) Stands: A Case Study. *Acta Silv. Lignaria Hung.* **2012**, *8*, 103–112. [CrossRef]
41. Roman, A.M.; Morar, I.M.; Truța, A.M.; Dan, C.; Sestraș, A.F.; Holonec, L.; Ioras, F.; Sestras, R.E. Trees, seeds and seedlings analyses in the process of obtaining a quality planting material for black locust (*Robinia pseudoacacia* L.). *Not. Sci. Biol.* **2020**, *12*, 940–958. [CrossRef]
42. Thapliyal, M.; Kaliyathan, N.N.; Rathore, K. Seed germination response of Indian wild pear (*Pyrus pashia*) to gibberellic acid treatment and cold storage. *Not. Sci. Biol.* **2021**, *13*, 11044. [CrossRef]
43. Lewak, S. Metabolic control of embryonic dormancy in apple seed: Seven decades of research. *Acta Physiol. Plant.* **2011**, *33*, 1–24. [CrossRef]

44. Borkowska, L. Patterns of seedling recruitment in experimental gaps on mosaic vegetation of abandoned meadows. *Acta Soc. Bot. Pol.* **2011**, *73*, 343–350. [CrossRef]
45. Basbag, M.; Aydin, A.; Ayzit, D. The Effect of Different Temperatures and Durations on the Dormancy Breaking of Black Locust (*Robinia pseudoacacia* L.) and Honey Locust (*Gleditsia triacanthos* L.) Seeds. *Not. Sci. Biol.* **2010**, *2*, 125–128. [CrossRef]
46. Mondoni, A.; Tazzari, E.; Zubani, L.; Orsenigo, S.; Rossi, G. Percussion as an effective seed treatment for herbaceous legumes (Fabaceae): Implications for habitat restoration and agriculture. *Seed Sci. Technol.* **2013**, *41*, 175–187. [CrossRef]
47. Zencirkiran, M.; Tümsavaş, Z.; Ünal, H. The Effects of Different Acid Treatment and Stratification Duration on Germination of Cercis siliquastrum L. Seeds. *Not. Bot. Horti Agrobot. Cluj-Napoca* **2010**, *38*, 159–163. [CrossRef]
48. Mirzaei, M.; Moghadam, A.R.L.; Ardebili, Z.O. The induction of seed germination using sulfuric acid, gibberellic acid and hot water in *Robinia pseudoacacia* L. *Int. Res. J. Appl. Basic Sci.* **2013**, *4*, 96–98.
49. Pârnuţă, G.S.E.; Budeanu, M.; Scărlătescu, V.; Marica, F.M.; Lalu, I.; Curtu, A.L. *Catalogul Naţional al Resurselor Genetice 462 Forestiere [National Catalogue of Forest Genetic Resources]*; Editura Silvică: Bucharest, Romania, 2011. (In Romanian)
50. De Micco, V.; Aronne, G. Morpho-Anatomical Traits for Plant Adaptation to Drought. In *Plant Responses to Drought Stress*; Springer: Berlin/Heidelberg, Germany, 2012; pp. 37–61.
51. Belokopytova, L.; Zhirnova, D.; Kostyakova, T.; Babushkina, E. Dynamics of moisture regime and its reconstruction from a tree-ring width chronology of Pinus sylvestris in the downstream basin of the Selenga River, Russia. *J. Arid. Land* **2018**, *10*, 877–891. [CrossRef]
52. Yang, B.; Peng, C.; Harrison, S.P.; Wei, H.; Wang, H.; Zhu, Q.; Wang, M. Allocation Mechanisms of Non-Structural Carbohydrates of *Robinia pseudoacacia* L. Seedlings in Response to Drought and Waterlogging. *Forests* **2018**, *9*, 754. [CrossRef]
53. Giuliani, C.; Lazzaro, L.; Calamassi, R.; Fico, G.; Foggi, B.; Lippi, M.M. Induced water stress affects seed germination response and root anatomy in *Robinia pseudoacacia* (Fabaceae). *Trees* **2019**, *33*, 1627–1638. [CrossRef]
54. Sestras, A.F. *Biostatistica si Tehnica Experimentala Forestiera: Manual Didactic*; Editura Academic Press: Cluj-Napoca, Romania, 2018.
55. Hammer, Ø.; Harper, D.A.; Ryan, P.D. PAST: Paleontological statistics software package for education and data analysis. *Palaeontol. Electron.* **2001**, *4*, 4–9.
56. Sestras, A.F. *Modele Statistice Aplicate în Cercetarea Horticola*; Editura Risoprint: Cluj-Napoca, Romania, 2019.
57. Rajora, O.P.; Mosseler, A. Challenges and opportunities for conservation of forest genetic resources. *Euphytica* **2001**, *118*, 197–212. [CrossRef]
58. Tozer, M.G.; Ooi, M. Humidity-regulated dormancy onset in the Fabaceae: A conceptual model and its ecological implications for the Australian wattle Acacia saligna. *Ann. Bot.* **2014**, *114*, 579–590. [CrossRef]
59. Safdar, M.E.; Wang, X.; Abbas, M.; Ozaslan, C.; Asif, M.; Adnan, M.; Zuan, A.T.K.; Wang, W.; Gasparovic, K.; Nasif, O.; et al. The impact of aqueous and N-hexane extracts of three Fabaceae species on seed germination and seedling growth of some broadleaved weed species. *PLoS ONE* **2021**, *16*, e0258920. [CrossRef] [PubMed]
60. Kheloufi, A.; Mansouri, L.; Aziz, N.; Sahnoune, M.; Boukemiche, S.; Ababsa, B. Breaking seed coat dormancy of six tree species. *Reforesta* **2018**, *5*, 4–14. [CrossRef]
61. Pedrol, N.; Puig, C.G.; López-Nogueira, A.; Pardo-Muras, M.; González, L.; Alonso, P.S. Optimal and synchronized germination of *Robinia pseudoacacia*, Acacia dealbata and other woody Fabaceae using a handheld rotary tool: Concomitant reduction of physical and physiological seed dormancy. *J. For. Res.* **2017**, *29*, 283–290. [CrossRef]
62. Kolbek, J.V.M.; Vetvicka, V. From history of Central European Robinia growths and its communities. *Zprávy České Bot. Společnosti* **2004**, *39*, 287–298.
63. Masaka, K.; Yamada, K. Variation in germination character of *Robinia pseudoacacia* L. (Leguminosae) seeds at individual tree level. *J. For. Res.* **2009**, *14*, 167–177. [CrossRef]
64. Zoghi, Z.; Azadfar, D.; Kooch, Y. The Effect of Different Treatments on Seeds Dormancy Breaking and Germination of Caspian Locust (*Gleditschia caspica*) Tree. *Ann. Biol. Res.* **2011**, *2*, 400–406.
65. Staska, B.; Essl, F.; Samimi, C. Density and age of invasive *Robinia pseudoacacia* modulate its impact on floodplain forests. *Basic Appl. Ecol.* **2014**, *15*, 551–558. [CrossRef]
66. Rédei, K.; Csiha, I.; Keserű, Z.; Végh Ágnes, K.; Gyori, J. The Silviculture of Black Locust (*Robinia pseudoacacia* L.) in Hungary: A Review. *South-East Eur. For.* **2011**, *2*, 101–107. [CrossRef]
67. Kleinbauer, I.; Dullinger, S.; Peterseil, J.; Essl, F. Climate change might drive the invasive tree *Robinia pseudacacia* into nature reserves and endangered habitats. *Biol. Conserv.* **2010**, *143*, 382–390. [CrossRef]
68. Jin, T.T.; Fu, B.J.; Liu, G.H.; Wang, Z. Hydrologic feasibility of artificial forestation in the semi-arid Loess Plateau of China. *Hydrol. Earth Syst. Sci.* **2011**, *15*, 2519–2530. [CrossRef]
69. Bouteiller, X.P.; Porté, A.J.; Mariette, S.; Monty, A. Using automated sanding to homogeneously break seed dormancy in black locust (*Robinia pseudoacacia* L., Fabaceae). *Seed Sci. Res.* **2017**, *27*, 243–250. [CrossRef]
70. Bouteiller, X.P.; Barraquand, F.; Garnier-Géré, P.; Harmand, N.; Laizet, Y.; Raimbault, A.; Segura, R.; Lassois, L.; Monty, A.; Verdu, C.; et al. No evidence for genetic differentiation in juvenile traits between Belgian and French populations of the invasive tree *Robinia pseudoacacia* . *Plant Ecol. Evol.* **2018**, *151*, 5–17. [CrossRef]
71. Mantovani, D.; Veste, M.; Freese, D. Black locust (*Robinia pseudoacacia* L.) ecophysiological and morphological adaptations to drought and their consequence on biomass production and water-use efficiency. *N. Z. J. For. Sci.* **2014**, *44*, 29. [CrossRef]