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Research Article

Responses of containerized seedlings of black locust (*Robinia pseudoacacia* L.) under the field conditions

Cristina DRĂGHICI[1]*, Raluca ENESCU[1], Adela HOBLE[2]*, Ioan V. ABRUDAN[1]

[1]*Transylvania University of Brașov, Faculty of Silviculture and Forest Engineering, Department of Silviculture, 1 Șirul Beethowen, 500123 Brașov, Romania; cristina.vacalie@unitbv.ro (*corresponding author); raluca.enescu@unitbv.ro; abrudan@unitbv.ro*
[2]*University of Agriculture Science and Veterinary Medicine, Faculty of Horticulture and Business in Rural Development, Department of Horticulture and Landscaping, 3-5 Calea Mănăștur, 400372 Cluj-Napoca, Romania; adela.hoble@usamvcluj.ro (*corresponding author)*

Abstract

This paper aimed to monitor the adaptation of one-year black locust seedlings in the field conditions. The seedlings of three Romanian provenances were previously produced in the containerized method under eight different substrate conditions (peat MKS 1, peat MKS 3, rendzina, rendzina + sand, dystric cambisol, dystric cambisol + sand, eutric cambisol and eutric cambisol + sand), and three water regimes (70%, 50% and 30%). The survival rate of seedlings and the main growth traits at the end of the first year after planting in the field were evaluated according to each type of treatment in which the seedlings were previously produced in containers. In field conditions, the growing traits of seedlings were not influenced by their provenance. The seedlings that were produced in the greenhouse in eutric cambisol + sand substrate, regardless of the water regime and provenance, were the shortest in the field conditions. The highest values of height were registered for the seedlings produced in peat MKS3 substrate under a medium watering regime. The seedlings that were produced in containers on dystric cambisol with sand substrate under high and medium water regimes had the best response in terms of survival in the field.

Keywords: greenhouse conditions; field conditions; provenance; seedling survival; seedling growth; substrate; water regimes

Introduction

All countries in Central and Western Europe have been producing containerized seedlings since the 90s. Since 2000 until now, Poland and the Baltic countries practice instead of producing nude root seedlings the production of 100% containerized seedlings, many European countries have been using fully mechanised containerisation for many years. Therefore, a series of studies have been carried out regarding both the identification of optimal conditions in which to produce containerized seedlings for various species and the

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advantage/disadvantages of their production and their subsequent survival, under the field conditions (Haase *et al.,* 2006; Veijalainen *et al.,* 2007; Tsakaldimi *et al.,* 2009; Thiffault *et al.* 2014; Jäärats *et al.,* 2016).

Of non-native species, *Robinia pseudoacacia* L. is the species with the widest use in Romania. Black locust seedlings grow rapidly and are mainly used for afforestation of degraded lands, in which only these can survive. Heaving a hard wood, it is preferred to be planted for future firewood. Since it grows quickly and finds favorable vegetation conditions in our country, it must be given the due importance in afforestation, being a fast-growing forest species of great economic importance (Abrudan, 2006).

In the context of climate change, water stress can even determine to some extent the mortality of seedlings (Wightman *et al.* 2018; Sung *et al.,* 2019). Yan *et al*. (2022) analyzes this phenomenon for a different gradient of precipitation in the Chinese Loess Plateau area. Although black locust is not traditionally grown in containers, due to the multiple advantages of producing containerized seedlings, a series of studies have been conducted on this species, as well (Ali, 1991; Kostopoulou *et al.,* 2010; Dini-Papanastasi *et al*., 2012; Dimitrova and Stoyanov, 2022).

Although containerized seedlings are more expensive than those with nude roots, they are the most recommended for afforestation of degraded lands. Containerized seedlings have the advantage of eliminating shock when transplanting and better withstand drought (Stowe *et al.,* 2010). As opposed to seedlings produced in the soil, which are able to plant after two years, containerized seedling is suitable for planting after only one year (Heiskanen and Rikala 2000; Heiskanen, 2004; Fan *et al*., 2004; South *et al*., 2005; Dominguez-Lerena *et al.,* 2006; Ferrini and Nicese, 2006). In the process of planting, the roots of the seedlings remain in a natural position and in an undisturbed connection with the soil or the physical environment in which they grew. The youngsters adapt easily and quickly to the new living conditions in the wooded land, being a possible solution against the high droughts and desertification of Romania. They also have a successful net superior regeneration at planting (approx. 95%), compared to seedlings with nude roots suffering from the stress of transplanting, being much more exposed to possible drought immediately after planting (Abrudan, 2006).

Potential problems with containerized seedlings

With the increasing use of technology to produce containerized seedlings, however, it seems that several shortcomings have been identified. However, a small but growing number of studies, both black locust and other species, suggest that container seedlings are not always more successful than those produced by direct sowing in the field.

Halter *et al*. (1993) found that in the case of an 11-year-old pine (*Pinus contorta*) culture set up with one-year-old containerized seedlings, they grew more slowly than those regenerated naturally in the same place. The first having different deformations of the root system, the most important being the lack of depth. McCreary (1995) examined the success of the blue oak (*Quercus douglasii*) culture founded with containerized seedlings and found that although survival in the first year was higher in their case than in the sowing direct, after that, survival was similar, and growth rates were significantly higher at sowings. Marshall and Gilman (1997) also examined the roots of a *Quercus virginiana* culture that was either grown in the field or produced in containers and found that the specimens grown in the field had the root system better developed both at a small depth (0 to 25 cm) and in deep horizons (75 to 100 cm), except intermediate depths. At the same time, only the specimens originally produced in containers suffered in the unirrigated plots. Also, Dimitrova and Stoyanov (2022) in a comparative study, obtained higher growths at the end of the first year, at black locust seedlings that were produced by sowings directly in the field, compared to containerized seedlings. The same thing was proved in our previous study (Drăghici *et al.,* 2024), when the black locust containerized seedlings produced in different substrate conditions and under different watering regimes, had smaller increases than those resulting from direct sowings in the field. Therefore, the attention from the last period is increasingly directed to the production of a high-quality planting material with the smallest investments (materials, seeds, substrate, quantity of water, etc.) for the afforestation of degraded land (Broshtilov *et al*., 1998; Gayton, 2001) but to adapt as easily as possible after transplanting, these possible potential problems must be considered.

The aim of this paper was to monitor the adaptation of one-year black locust seedlings in the field (natural conditions). The seedlings with three Romanian provenances were previously produced in containers under different substrate conditions and water regimes (70%, 50% and 30%). The objectives had been established to determine their survival rate and the main dimensional parameters at the end of the first year after planting in the field, depending on each type of treatment in which they were previously produced in containers.

Materials and Methods

Study site

This study was conducted on an experimental field, of the Research and Development Institute of the Transylvania University of Brasov. The area is situated in the central part of Romania (Brasov area – Figure 1) and is characterized by a temperate continental climate with the average annual temperature being around 7.8 °C, the average altitude is 625 m, the average annual rainfall value of 742.2 mm, annual evapotranspiration 601 mm, relative air humidity 71% (Țîștea *et al*., 1961).

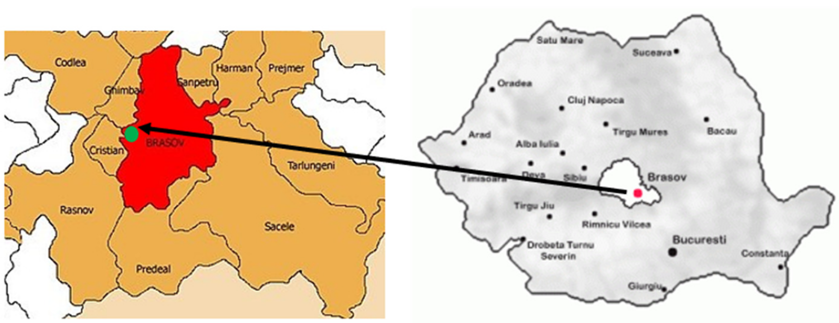


Figure 1. Geographical location of the study site

In the experimental field two soil profiles (P1 and P2) were placed for a better overview about soil properties (Table 1). The soil has a weak acid reaction, and it is eubasic. According to the content from the A horizon it is moderately humiferous. The soil texture is clayey-sandy loamy until 40 cm deep and then the proportion of the sand increases.

Table 1 presents the main characteristics of soil determined according to the national and international standards (using three replicates for each substrate type) in the Soil Science Laboratory from the Faculty of Silviculture.

The physical property (granulometric composition – soil texture) was determined through wet sieving and sedimentation in a water column [ISO11277, 1998]. The soil pH was determined by potentiometric method in water suspension [ISO 10390, 2005]. The modified Walkley–Black method has been used for total carbon content [FAO, 2019].

For the bases exchange capacity (SB) and for the hydrogen exchange capacity (SH) was used Kappen method [STAS7184/12-88, 1988]. The soil treatment was made with hydrochloric acid solution for SB and for SH with potassium acetate solution. The total cation exchange capacity and the base saturation degree were determined using two specific formulas (STAS 7184/12-88, 1988).

Table 1. The characteristics of soil from field condition

Indices		Soil profile					
		P1			P2		
		0-10 (cm)	30-40 (cm)	70-80 (cm)	0-10 (cm)	30-40 (cm)	70-80 (cm)
Coarse (%)		56.60	62.80	81.60	58.70	64.70	73.90
Silt (%)		19.20	16.70	6.10	15.30	11.80	8.00
Clay (%)		24.20	20.50	12.30	26.00	23.50	18.10
Texture		Clayey-sandy loam	Clayey-sandy loam	Loamy sand	Clayey-sandy loam	Clayey-sandy loam	Sandy loam
pH		6.63	6.80	6.83	6.06	6.40	6.53
Organic matter content (%)		5.43	4.85	1.68	4.64	3.75	0.92
Total carbon content (%)		3.15	2.81	0.98	2.69	2.18	0.53
Bases exchange capacity	(me/100 g soil)	61.6	51.2	24.8	48.8	43.2	27.2
Hydrogen exchange capacity		5.2	4.0	2.4	7.2	4.8	2.8
Total cation exchange capacity (me/100 g soil)		66.8	55.2	27.2	56.0	48.0	30.0
Base saturation degree (%)		92.2	92.8	91.2	87.1	90.0	90.7

Statistical analysis

The height of the seedlings was measured first time in April (after planting) and the second time in October separately on water regime and was processed using one-way analysis of variance (ANOVA). The measured parameters of the seedlings (height, diameter, number of branches and height to the first branch) were compared to the mean values using the Duncan test (significant differences, $p<0.05$ were marked with different letters).

Also, was made a series of correlations between diameter of seedlings, height to the first branch and the number of branches stratified on the type of substrate and of the type of provenance and then without being stratified on each treatment. All the statistical analyses were performed with Microsoft Excel and Statistica v.8 softs.

Biological material and experimental procedures

The biological material was represented by one year old containerized black locust seedlings, produced in a greenhouse at Sânpetru Educational and Research Base, Transylvania University of Brasov (Drăghici *et al*. 2024).

The seeds were collected from the Romanian seed sources, which are included in National Catalogue of Forest Genetic Resources: (Pârnuță *et al*. 2011): Hanu Conachi (South-East of Romania), Bucharest (South of Romania) and Săcueni (North-West of Romania) and eight different types of substrate were used to seedlings production (peat MKS 1 (S1), peat MKS 3 (S2), rendzina(S3), rendzina + sand (1:2) (S4), dystric cambisol (S5), dystric cambisol + sand (1:2) (S6), eutric cambisol (S7) and eutric cambisol + sand (1:2)(S8)) under three distinct irrigation regimes (high watering (70% of available water capacity – AWC) (A1); medium watering (50% of AWC) (A2); low watering (30% of AWC)(A3)). This was the subject of a previous study (Drăghici *et al*., 2024).

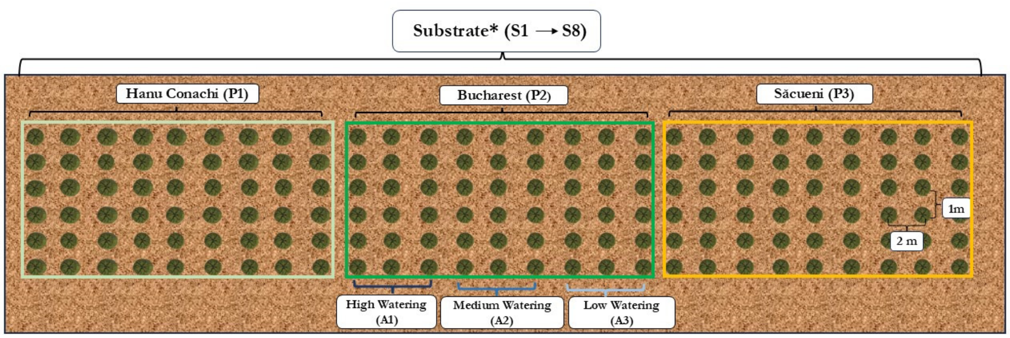
From October 2023 until they were planted, the seedlings were kept outside, in the nursery of the Sânpetru Educational Base (Figure 2- A).

In the spring of 2024, 18 more vigorous seedlings were selected from each treatment and planted on 1[st] of April, 2024, in the experimental field of the Research and Development Institute of Transylvania University of Brasov (Figure 2 - B, C and D), 18×8×3×3 (18 seedlings for each treatment – repeats; 8 types of substrates

(S1-S8); 3 seed sources of provenance (P1-P3); 3 watering regimes (A1-A3) resulting in 1296 measurements of the seedling growth rate for April and October. The first measurement of the height and base diameter was made at planting, and the second one, in October, at the end of the growing season. In October, along with seedling height and diameter, the number of branches and height to the first branch were also determined.

The seedlings were placed in a randomized block, depending on the treatment previously applied, in a planting scheme of 1×2 m (Figure 2 - E). The same principle was applied to each of the eight substrate types.

(A) (B) (C) (D)



(E)

Figure 2. General aspects regarding field experiment; (A) Sânpetru Educational Base nursery; (B) The experimental field of Brasov Research Institute; (C) Seedlings after planting; (D) Seedlings in October 2024; (E) Experimental diagram for field black locust seedlings according to randomized complete block design

* S1 – Peat MKS 1; S2 – Peat MKS 3; S3 – Rendzina; S4 – Rendzina + sand; S5 – Dystric cambisol; S6 – Dystric cambisol+sand; S7 – Eutric cambisol; S8 – Eutric cambisol+sand

Results

Seedling growth characteristics

The analysis of variance has been processed for seedlings diameter and height (Table 2). For the diameter were obtained significant differences only for substrate at the end of growing season. Regarding the height of the seedlings, we can observe a bigger number of significant differences – two in April (substrate and watering regime) and two in October (for the same parameters).

Table 2. The analysis of variance for seedlings diameter and height

Factors	F-value			
	Diameter		Height	
	April	October	April	October
Substrate (S)	1,532	**6,941*****	**48.721*****	**34.958*****
Seed source of provenance (P)	0,997	1,233	45.879	0.116
Watering regime (A)	1,164	0,359	**20.527*****	**23.969*****

* - $\alpha \leq 5\%$; ** - $\alpha \leq 1\%$; *** - $\alpha \leq 0,1\%$

The height means values obtained for the seedlings as an interaction effect between provenance and substrate (Table 3). Both in April at planting and in October at the end of the growing season, the tallest seedlings were those from Săcueni provenance (P3), produced in the greenhouse in peat MKS1 substrate (S1). The lowest increases in height were maintained on the same substrate, also for Săcueni provenance (P3) in October, but in April they belong to the Bucharest provenance (P2), for the same substrate

Table 3. The analysis of significance between provenance and substrate on height (cm) of black locust seedlings

Provenance*	Substrate*	April	October
P1	S1	62.78^{cd}	43.15^{efg}
	S2	59.81^{def}	61.80^{abcd}
	S3	52.24^{ijk}	52.01^{cdef}
	S4	54.61^{ghij}	64.43^{abc}
	S5	46.83^{lm}	58.96^{abc}
	S6	52.83^{hijk}	56.54^{abc}
	S7	56.41^{efghi}	67.35^{ab}
	S8	50.19^{jkl}	40.31^{fgh}
P2	S1	68.11^{ab}	56.31^{abc}
	S2	62.30^{cd}	59.94^{abcd}
	S3	54.87^{ghi}	61.73^{abcd}
	S4	59.20^{defg}	57.20^{abc}
	S5	49.56^{kl}	67.57^{ab}
	S6	55.30^{fghi}	55.43^{cdef}
	S7	56.00^{efghi}	49.11^{def}
	S8	43.44^{m}	32.80^{gh}
P3	S1	71.80^{a}	54.74^{cdef}
	S2	62.33^{cd}	72.76^{a}
	S3	60.13^{cde}	63.50^{abc}
	S4	62.78^{cd}	55.83^{abc}
	S5	53.74^{hijk}	56.39^{abc}
	S6	57.35^{efgh}	60.81^{abcd}
	S7	64.41^{bc}	54.98^{cdef}
	S8	55.56^{efghi}	30.31^{h}
SD		3.99 - 4.96	10.94 - 13.62

The means followed by different letters are significantly different according to Duncan's test ($p < 0.05$)

* P1 – Hanu Conachi; P2 – Bucharest; P3 – Săcueni

* S1 – Peat MKS 1; S2 – Peat MKS 3; S3 – Rendzina; S4 – Rendzina + sand; S5 – Dystric cambisol; S6 – Dystric cambisol+sand; S7 – Eutric cambisol; S8 – Eutric cambisol+sand

Data presented below show the mean heights as interaction effect between provenance and water treatments (Table 4).

Table 4. The analysis of significance between provenance and water regimes on height (cm)

Provenance*	Water treatment*	April	October
P1	A1	55.02[d]	56.36[a]
	A2	54.09[de]	55.98[a]
	A3	54.28[de]	54.37[a]
P2	A1	58.00[c]	55.15[a]
	A2	58.17[c]	56.72[a]
	A3	52.12[e]	53.17[a]
P3	A1	64.01[a]	56.76[a]
	A2	61.55[b]	57.85[a]
	A3	7.47[c]	53.89[a]
SD		2.45 - 2.88	6.45 - 7.58

The means followed by different letters are significantly different according to Duncan's test ($p < 0.05$)

* P1 – Hanu Conachi; P2 – Bucharest; P3 – Săcueni

* A1 – High watering; A2 – Medium watering; A3 – Low watering

Provenance Bucharest (P2) presented the lowest growth, both in April and in October, under the A3 water regime (low watering regime), with significant differences only in April. In October there were no significant differences between the origins, regardless of the water regime applied previously, to produce containerized seedlings. The highest increase was recorded by the seedlings from Săcueni, but for different water regimes (in April – high watering regime, in October - medium watering regime).

The interaction effect between substrate and water treatments is rendered as mean hights (Table 5). Seedlings in eutric cambisol + sand (1:2) substrate are the shortest regardless of the water treatment, in October, with significant differences from all other regimes. The tallest seedlings with medium watering regime were in peat MKS3 substrate (S2).

The mean heights of seedlings recorded as effects of seed provenance on growth. At the first measurement (in April at planting) but also in October, seedlings from Săcueni presented the highest vigour for height, but in October there were no significant differences compared to the other two (Table 6).

The mean heights of seedlings are recorded as effects of substrate on the growth (Table 7). Peat MKS 1 has the greatest influence at the first measurement (April). At the second measurement (October), the greatest increases are recorded for the treatments with peat MKS 3. At both measurements the lowest increases were on the S8 substrate (eutric cambisol + sand).

The results in regard to seedlings diameter (mm) measurements are presented in Figure 3. Depending on the watering treatment, at the end of the growing season, there were no significant differences between the three applied regimes and neither according to the origin, except for the origin of Hanu Conachi (P1). The substrate had a greater influence on the growth of seedlings in diameter, the highest values recorded in October for seedlings that were produced on eutric cambisol (S7).

Table 5. The analysis of significance between substrate and water regimes on height (cm)

Substrate*	Water treatments*	April	October
S1	A1	72.28[a]	50.78 [cd]
	A2	70.83[a]	55.02[bcd]
	A3	59.57[cde]	48.41[d]
S2	A1	65.02[b]	63.41[abc]
	A2	56.46 [efgh]	71.19[a]
	A3	62.96[bc]	59.91[abcd]
S3	A1	57.35[defg]	61.85[abc]
	A2	55.33[efgh]	56.53[bcd]
	A3	54.56[fgh]	58.87[abcd]
S4	A1	58.41[def]	59.76[abcd]
	A2	61.48[bcd]	58.63[abcd]
	A3	56.70[efgh]	59.07[abcd]
S5	A1	53.61[gh]	64.59[ab]
	A2	52.07 [hi]	61.22[abc]
	A3	44.44j	57.11[bcd]
S6	A1	57.83[defg]	57.85[bcd]
	A2	55.33[efgh]	62.00[abc]
	A3	52.31[hi]	52.93[bcd]
S7	A1	59.31[cde]	53.56[bcd]
	A2	59.50[cde]	56.39[bcd]
	A3	58.00[defg]	61.50[abc]
S8	A1	48.28[ij]	36.94[e]
	A2	52.48[hi]	33.81[e]
	A3	48.43[ij]	32.67 [e]
SD		4 - 4.98	10.53 - 13.11

The means followed by different letters are significantly different according to Duncan's test ($p < 0.05$)
*S1 – Peat MKS 1; S2 – Peat MKS 3; S3 – Rendzina; S4 – Rendzina + sand; S5 – Dystric cambisol; S6 – Dystric cambisol+sand; S7 – Eutric cambisol; S8 – Eutric cambisol+sand
*A1 – High watering; A2 – Medium watering; A3 – Low watering

Table 6. The analysis of significance of seed source and provenance on height (cm) of black locust seedlings

Provenance*	April	October
P1	54.46[c]	55.57[a]
P2	56.10[b]	55.01[a]
P3	61.01[a]	56.17[a]
SD	1.41 - 1.48	3.87 - 4.07

The means followed by different letters are significantly different according to Duncan's test ($p < 0.05$)
* P1 – Hanu Conachi; P2 – Bucharest; P3 – Săcueni

Table 7. The analysis of significance of substrate on height (cm) of black locust seedlings

Substrate*	Seedlings height (cm)	
	April	October
S1	67.56^{a}	51.40^{b}
S2	61.48^{b}	64.83^{a}
S3	55.75^{d}	59.08^{a}
S4	58.86^{c}	59.15^{a}
S5	50.04^{e}	60.98^{a}
S6	55.16^{d}	57.59^{ab}
S7	58.94^{c}	57.15^{ab}
S8	49.73^{e}	34.48^{c}
SD	2.37 - 2.75	6.85 - 7.95

The means followed by different capital letters are significantly different according to Duncan's test ($p < 0.05$)
* S1 – Peat MKS 1; S2 – Peat MKS 3; S3 – Rendzina; S4 – Rendzina + sand; S5 – Dystric cambisol; S6 – Dystric cambisol+sand; S7 – Eutric cambisol; S8 – Eutric cambisol+sand

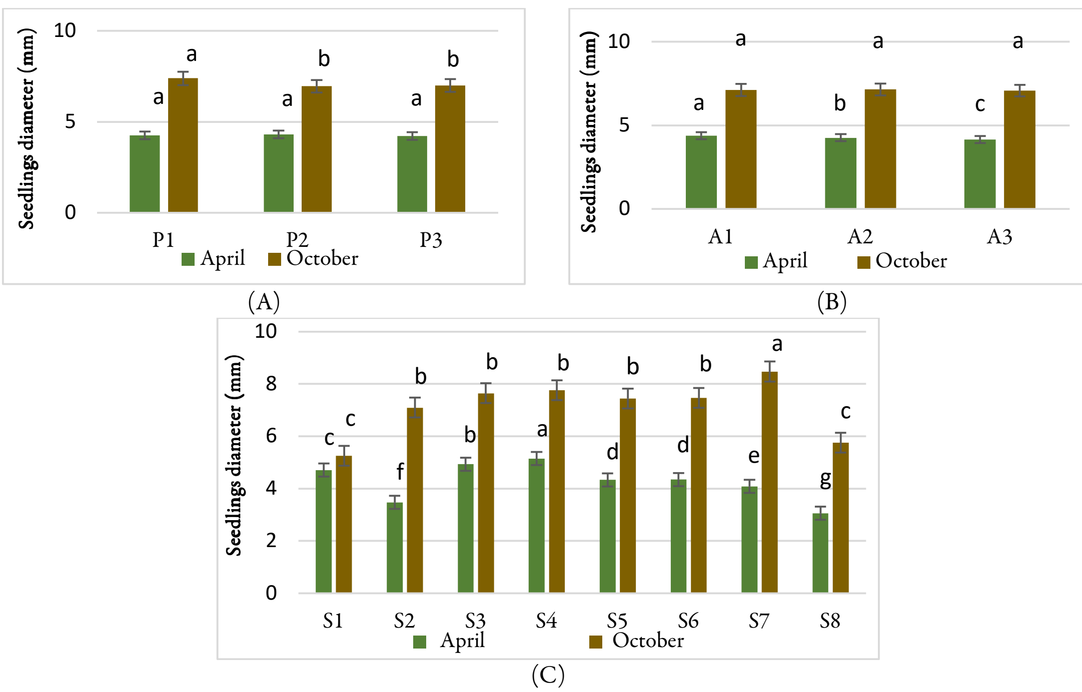


Figure 3. Seedlings diameter (mm), influence by the provenance (A), watering regimes (B) and substrate (C) (bars = mean diameter, error bars = standard error)
* S1 – Peat MKS 1; S2 – Peat MKS 3; S3 – Rendzina; S4 – Rendzina + sand; S5 – Dystric cambisol; S6 – Dystric cambisol+sand; S7 – Eutric cambisol; S8 – Eutric cambisol+sand
* P1 – Hanu Conachi; P2 – Bucharest; P3 – Săcueni
* A1 – High watering; A2 – Medium watering; A3 – Low watering

Seedling survival

The results regarding the loss of seedlings according to provenance and water regime for all eight substrate types (S1-S8) (Figure 4). For each substrate variant, there were initially 162 seedlings (18 repetitions, three provenances, three watering regimes).

The seedlings survival showed significant losses for the variant with the peat MKS 1 (S1 -Hanu Conachi provenance, high watering regime) and for the variant with eutric cambisol (S7 -Bucharest provenance, high watering regime) as well.

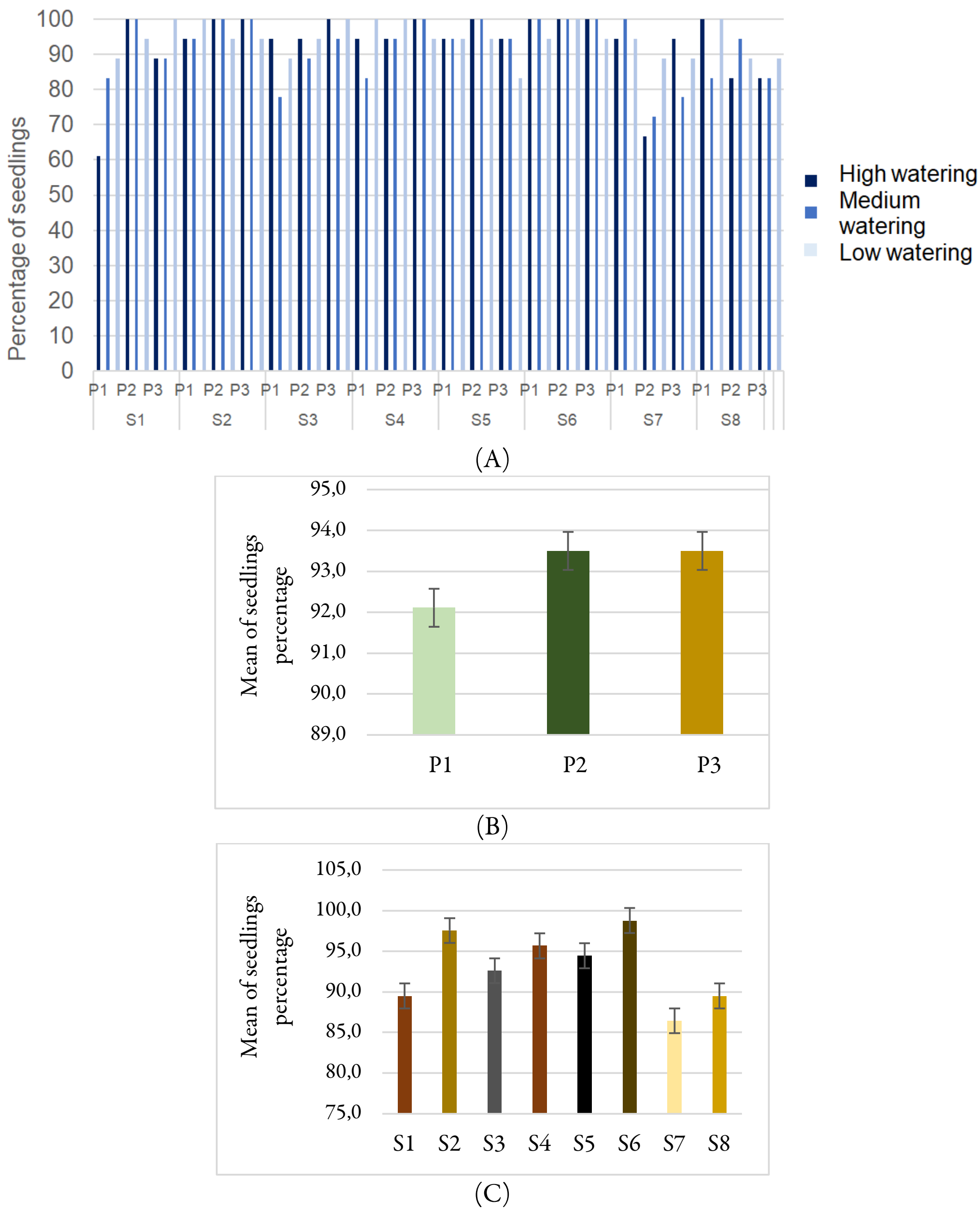


Figure 4. Seedlings survival influenced by the substrate type, provenance and watering regime SxPxA (A), influenced by provenance (B), influenced by the substrate type (C) (bars = mean percentage, error bars = standard error)

* S1 – Peat MKS 1; S2 – Peat MKS 3; S3 – Rendzina; S4 – Rendzina + sand; S5 – Dystric cambisol; S6 – Dystric cambisol+sand; S7 – Eutric cambisol; S8 – Eutric cambisol+sand

* P1 – Hanu Conachi; P2 – Bucharest; P3 – Săcueni

* A1 – High watering; A2 – Medium watering; A3 – Low watering

The origin did not influence the percentage of lost seedlings until the end of the first growing season (October 2024) (Figure 4). In contrast, the data presented in figure 4C, indicate a significant influence in terms of the number of seedlings that have survived, taking into account only the variable substrate. The highest number of surviving seedlings belong to the MKS 3 substrate (S2).

From the interaction of the substrate with the applied watering regime (table 8) one can see that in the case of dystric cambisol + sand (1:2) substrates (B6), both at high water regime and for medium watering, no seedling was lost. The fewest seedlings survived on the MKS 1 substrate (S1) and on the eutric cambisol substrate (S7).

Table 8. Seedlings survival (no.) influenced by the substrate type and watering regime

Substrate*	Seedlings survival (number)		
	Water treatments		
	A1*	A2*	A3*
S1	15.00[d]	16.33[abcd]	17.00[abcd]
S2	17.67[ab]	17.67[ab]	17.33[abc]
S3	17.33[abc]	15.67[bcd]	17.00[abcd]
S4	17.33[abc]	16.67[abcd]	17.33[abc]
S5	17.33[abc]	17.33[abc]	17.00[abcd]
S6	18.00[a]	18.00[a]	17.33[abc]
S7	15.33[cd]	15.00[d]	16.33[abcd]
S8	16.00[abcd]	15.33[cd]	16.67[abcd]
SD	1.75 - 2.11		

* S1 – Peat MKS 1; S2 – Peat MKS 3; S3 – Rendzina; S4 – Rendzina + sand; S5 – Dystric cambisol; S6 – Dystric cambisol+sand; S7 – Eutric cambisol; S8 – Eutric cambisol+sand

* A1 – High watering; A2 – Medium watering; A3 – Low watering

Correlations between measured characteristics of the seedlings

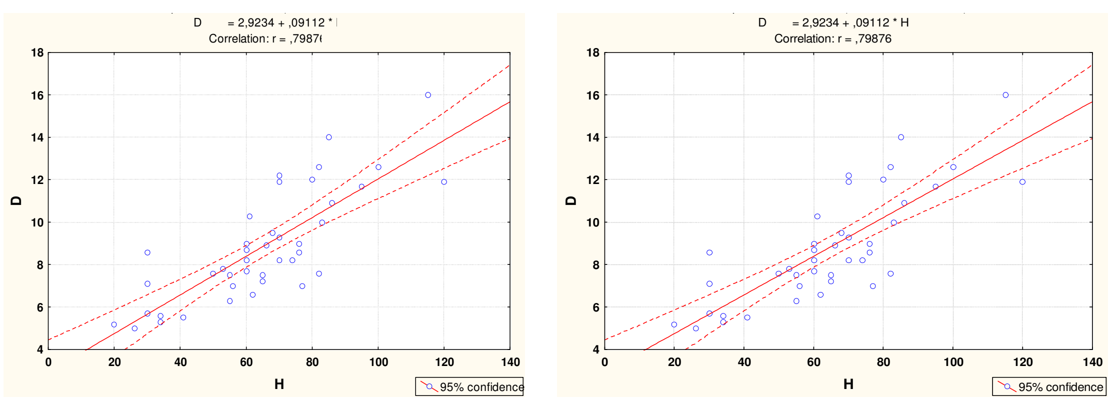
The correlation between measured characteristics of the seedlings that have been stratified on type of substrate and type of provenance are presented in Table 9. In the table was kept just those correlations that were significant from a statistical point of view. The biggest values of correlation coefficient were obtained between the height of the seedlings and the diameter for the seedlings who grew up in the rendzina substrate from the Bucharest provenance (0.88) and for Eutric cambisol substrate from the same provenance (0.80).

Table 9. Correlations between the measured characteristics of the seedlings influenced by substrate and provenance

Substrate*	Provenance*	Measured characteristics	Diameter of seedlings	Number of branches	Height to the first branch
S1	P2	The height of the seedlings	0.11	0.42	0.28
S2	P1	The height of the seedlings	0.58	0.40	0.18
		Diameter of seedling	-	0.36	0.21
		Number of branches	-	-	-0.28
	P2	The height of the seedlings	0.39	0.58	-0.08
		Diameter of seedling	-	0.29	-0.20
		Number of branches	-	-	-0.34
	P3	The height of the seedlings	0.70	0.50	0.19
S3	P1	The height of the seedlings	0.76	0.34	0.43
		Diameter of seedling	-	0.41	0.36
		Number of branches	-	-	0.03
	P2	The height of the seedlings	0.88	0.41	0.55
		Diameter of seedling	-	0.46	0.45
		Number of branches	-	-	-0.25
	P3	The height of the seedlings	-0.37	-0.04	0.40

S4	P1	The height of the seedlings	0.60	0.60	0.45
		Diameter of seedling	-	0.55	0.03
	P2	The height of the seedlings	0.61	0.44	0.36
		Diameter of seedling	-	0.33	0.41
	P3	The height of the seedlings	-0.25	0.60	0.23
S5	P1	The height of the seedlings	0.79	0.33	0.29
		Diameter of seedling	-	0.43	0.20
	P2	The height of the seedlings	-0.11	0.25	0.28
	P3	The height of the seedlings	0.55	0.28	0.47
S6	P1	The height of the seedlings	0.56	0.55	0.34
		Diameter of seedling	-	0.50	0.11
	P2	The height of the seedlings	0.70	0.51	0.38
		Diameter of seedling	-	0.41	0.10
	P3	The height of the seedlings	0.68	0.50	0.34
		Diameter of seedling	-	0.51	0.02
S7	P1	The height of the seedlings	-0.24	0.43	0.43
		Diameter of seedling	-	0.23	-0.09
	P2	The height of the seedlings	0.80	0.37	0.45
		Diameter of seedling	-	0.64	0.23
	P3	The height of the seedlings	0.74	0.58	0.51
		Diameter of seedling	-	0.60	0.32
S8	P1	The height of the seedlings	0.77	0.52	0.68
		Diameter of seedling	-	0.54	0.53
		Number of branches	-	-	0.33
	P2	The height of the seedlings	0.57	0.33	0.49
		Diameter of seedling	-	0.48	0.35
		Number of branches	-	-	0.59
	P3	The height of the seedlings	0.34	0.44	0.56
		Diameter of seedling	-	0.37	0.50

For the highest values of the correlation coefficient (0.88 - between height and diameter from S3 substrate and P2 provenance, respectively 0.80 – between height and diameter from S7 substrate and P2 provenance) were made two charts for a better visualisation of the correlation (Figure 5).



(A) (B)

Figure 5. Correlation values between diameter (D) and height (H) for S3 substrate (rendzina) and P2 provenance (Bucharest)(A) and for S7 substrate (eutric cambisol) and P2 provenance (Bucharest) (B)

Finally, the correlation between the main parameters measured in October (seedling height, seedling diameter, number of branches per stem, stem height up to the first branch) was also achieved (Table 10). The data were not stratified on each treatment, obtaining a significant positive correlation between the height of the seedlings and diameter, number of branches and height up to the first branch. A significant positive correlation between diameter and number of branches has also been realised.

Table 10. Correlations between the analysed characteristics of the seedlings

Seedlings characteristics	Value of correlation coefficient		
	Diameter of seedlings	Number of branches	Height to the first branch
The height of the seedlings	0.17*	0.34*	0.38*
Diameter of seedlings	-	0.11*	0.06
Number of branches	-	-	0.03

*Significant, $p<0.05$

Discussion

Provenance

Our results regarding *R. pseudoacacia* seedlings growth characteristics have shown that there were not significant differences between the black locust origins for the main elements of seedlings growth, at the end of the growing season, from their planting in the field. Although, in April, at the first measurement made when planting them in the field, both in terms of height and in terms of diameter, there were significant differences between the three origins, after their development in natural conditions for growing season, the trend was not preserved, the differences in growth being insignificant. The place of harvest of the seeds (provenance) and the genotype, generally influence their germination capacity and the quality of the seedlings (Moshki and Lamersdor, 2011; Roman *et al.*, 2020; Roman *et al.,* 2022a,b), this has not been proven in our research one year after planting of the seedlings. Significant differences in growth, regarding the source, were also obtained between five different provenances by Roman *et al*. (2022), seedlings from Voievodeni and Trestia (Romania country) origin having the largest increases in height and diameter. Also, differences between the growth parameters of the clones have been proven in the research of Tsanov *et al*. (1992), who tested 6 populations of black locust in Bulgaria, two of them recorded the largest increases. Dimitrova *et al.* (2022) in the study of one-year-old seedlings from seven sources, obtained the best growths for Appalachia, Jaszkiseri and Riyhovo-7, origin, for seedlings from direct sowing in the field, similar to our previous study (Drăghici *et al*. 2024).

Substrate

The highest values of the heights were reached in October at seedlings that were produced in the greenhouse on the peat MKS 3 substrate, but there were no significant differences other than the MKS 1 substrate and eutric cambisol + sand (1:2) substrate. In April, the biggest heights are recorded on the MKS 1 substrate. However, the same trends were not observed at the diameter, the best increases this time being made for seedlings that were produced on eutric cambisol (S7), with significant differences from all other types. Peat mixed with vermiculite (3:1 and 2:1) proved to be the most suitable substrate both in terms of growth and subsequent survival of seedlings (Lin *et al*., 2010.

Therefore, given that the substrate on which seedlings are produced can influence both their growth and their survival rate after transplantation (Dini-Papanastasi *et al*., 2012; Iakovoglou *et al*., 2012) however, in our study the survival rate for seedlings produced on eutric cambisol substrate (S7) was the lowest, but with insignificant differences in comparison to all others. At the opposite end, the maximum survival rate was recorded for dystric cambisol + sand substrate (S6) for high and medium watering (A1 and A2).

In another comparative study, the growth medium also produced significant effects mainly at the growth stage of seedlings, and the origin of the seeds influenced more but only their subsequent survival (Dini-Papanastasi *et al.*, 2012), one of the reasons why it is interesting to continue this research.

Watering regimes

So, besides the type of substrate, the growth of black locust is also influenced by the amount of water available (Vitkova *et al.*, 2015). In the case of seedlings grown in containers on the peat MKS 3 substrate (S2), the best increases in height in October were recorded for those with medium watering regime (A2). Taking into account only the water variable, even if in April both between the heights and between the diameter of the seedlings that were previously produced in containers under the three watering regimes (Ombódi *et al.*, 2022; Wang *et al.*, 2023; Liu *et al.*, 2023), there were significant differences, at the end of the first growing season, in October, both height increases and diameters differences were insignificant. The degree of adaptability of black locust to different levels of humidity and the morpho-physiological changes that occur (Yan *et al.*, 2010; Zhang *et al.*, 2011; Liu *et al.,* 2013; Norouzi Haroni and Tabari Koochksaraee, 2015; Liu *et al.,* 2021; Yan *et al.*, 2022; Szyp-Borowska *et al.*, 2022) they are very important in the current climate change context because many regions of the world are expected to be affected by increased desertification and this is one of the species commonly used in climate change projects afforestation of degraded lands. The frequency of irrigation (Ranney *et al.*, 1990; Li *et al.* 2022) could better facilitate the survival (Elliott and Swank, 1994) and growth (Guse *et al.,* 2011; Mantovani *et al.,* 2014; 2015) of *Robinia pseudoacacia* seedlings and possibly promote the process of vegetation restoration in the future context of global climate change (Li *et al.*, 2022).

In our study, seedlings that were produced in containers on dystric cambisol + sand substrate (B6) both at high and medium water regime, have been adapted, the best in terms of survival, until the end of the first season in the field.

Conclusions

At the end of the growing season, there were no significant differences in the main growth traits of seedlings regarding provenance.

The substrate is essential to produce black locust seedlings, which subsequently influences their growth and survival after transplantation. In field conditions, at the end of the first growing season, the tallest seedlings were those obtained one year before in peat MKS 1 substrate (S1) and under a high watering regime. Also, the seedlings originally produced in the greenhouse on the peat MKS 3 substrate and under the medium watering regime (A2) had significant increases in the field. When the substrate was correlated with the place of harvest of seeds, the maximum values for the same substrate types were preserved, both in April and in October.

The smallest increases in height (both in April and October) were registered at the same origin (Bucharest) under the low watering regime (A3). Although the first measurement showed significant differences compared to all other variants, in October all the differences were non-significant.

In the case of Hanu Conachi provenance, even though the field experiment used the most vigorous seedlings, which were obtained in peat MKS 1 substrate (S1) and with a high watering regime (A1), survived the fewest seedlings at the end of the growing season. The highest survival rate was determined for the seedlings obtained in dystric cambisol and sand substrate (S6) with high and medium watering regimes (A1 and A2), all seedlings maintaining their vitality.

This work is a starting point for future research on identifying the best technique for producing black locust seedlings and monitoring the degree of adaptation of containerized seedlings under the field conditions as well as the identification of the extent to which its origin, substrate or watering regime from the greenhouse influence their survival and subsequent growth.

Authors' Contributions

Conceptualization: CD and IVA; Data curation: CD and RE; Formal analysis: CD and RE; Funding acquisition: IVA; Investigation: CD; Methodology: CD and AH; Project administration: CD and IVA; Resources: IVA; Software: CD, RE and AH; Supervision: IVA and AH; Validation: IVA and AH; Visualization: RE and CD; Writing - original draft: CD and RE; Writing - review and editing: AH.

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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