Effects of seed origin, growing medium and mini-plug density on early growth and quality of black locust (*Robinia pseudoacacia* [L.]) seedlings

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ABSTRACT: The objective of this study was to identify optimal growing conditions for the production of high-quality mini-plug seedlings of black locust (*Robinia pseudoacacia* [L.]). Two seed origins (Greek and Hungarian), two growing media (enriched peat and stabilized medium) and four mini-plug densities (975; 1,460; 1,820 and 3,500 mini-plugs·m^{-2}) were tested in two phases, in growth chambers and in a nursery after transplanting the mini-plugs into larger containers. Measurements included seedling survival, growth traits (root length, shoot height, leaf area, root dry mass, shoot dry mass, root/shoot ratio) and root growth potential (RGP). In addition, the ratio of variable to maximal fluorescence (F_v/F_m) and the effective quantum yield of photosystem II ($\Delta F/F'_m$) were measured. It was found that growing medium produced significant effects mainly in the first phase and seed origin in the second phase while mini-plug density was important in both phases. The F_v/F_m ratio was found to be significantly affected by growing medium while the effective quantum yield was influenced by density. Early survival of seedlings was closely correlated with fluorescence and growth traits (particularly with plant height) suggesting that these parameters might be useful for the grading of black locust seedlings prior to transplanting.

Keywords: mini-plug seedlings; growth traits; survival; mini-plug transplants; forest restoration

In the last decades there has been an increasing demand for plantation forestry to expand by using a much broader range of species so that a wider set of sites are exploited or restored for economic and conservation purposes (Weber 2005). Among them are the fast growing species (e.g. poplars, eucalypts, black locust, etc.). According to Ciccarese (2005), industrial plantations of these species currently meet a significant portion of the local and international needs for timber and energy.

Success of reforestation projects depends very much on the climatic and edaphic conditions that the established seedlings experience. Adverse environmental conditions may cause stress and subsequently result in damage at the biochemical, physiological and morphological levels (Margolis, Brand 1990; Andreucci et al. 2006). In such conditions, careful selection and planting of high-quality stock are important for ensuring high survival and growth of seedlings in the field (Radoglou 2001). In addition, planting stock of high quality can alleviate the negative impact of unfavourable environmental conditions and result in successful seedling establishment while poor-quality planting stock may result in an unsuccessful establishment even under favourable environmental conditions (Radoglou et al. 2009).

Containerized transplants grown in trays with small cells (mini-plugs) have the potential for reducing post-planting stress. Mini-plugs are very small container plants grown in cells less than 33 cm^3 in volume, usually filled up with a stabilized

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growing medium. In contrast to standard container seedlings, mini-plugs are fully extractable with a dimensionally stable root plug that tolerates transplanting within a relatively short growing period (3–4 months) (Landis 1990). Mini-plugs are used in two distinct stock types: container-to-bare-root transplants and container-to-container transplants (Riley, Steinfield 2005). Their advantages are an almost 100% yield, high plant density per production area, maximum use efficiency of seeds or cuttings and improved stock quality (Landis 2007).

Black locust (*Robinia pseudoacacia* [L.]) is a nitrogen-fixing leguminous fast growing tree, native to southeastern North America, but also naturalized in the temperate regions of North America, Europe and Asia. It is a multipurpose species suitable for lumber, poles, fuel, land reclamation, beekeeping, forage and wood fibre (Barrett et al. 1990; Bongarten et al. 1992). It is also extensively used for erosion control and reclamation of surface mines (Boring, Swank 1984). Recently, black locust has become important in plantation forestry worldwide. In Europe, for example, plantation investment companies are promoting the species for the afforestation of grasslands or agricultural lands (Nobis 2008; Anonymous 2009). Specifically in Hungary, the species is the most important stand-forming tree species occupying approximately 20% of the forested lands and providing 18% of the country's annual timber production (Redei et al. 2002). Also, in Greece, when the EU Directive on the Afforestation of Farmlands for Timber Production (No. 2080/1992 and 1257/99) was initiated in 1993, large areas of private-owned plantations were established with this species because it is considered fast growing and well adapted to marginal sites (Dini-Papanastasi 2004). As a consequence, the demand for black locust planting stock is high, necessitating the development of a seedling quality assessment protocol specific for this particular species (Wilson, Jacobs 2006).

Traditionally, black locust plantations are established by bareroot seedlings produced in conventional nurseries on a large scale (Redei et al. 2008). Bareroot plants, though, have some disadvantages; they can be produced in a certain period of the year, need to be immediately transplanted, and their transportation to remote planting sites is costly. On the other hand, black locust has not been tested for its amenability to container production, especially to mini-plug technology. The objective of this paper was to identify suitable growing conditions for the production of high-quality mini-plug seedlings of black locust so that appropriate cultivation protocols could be developed.

MATERIAL AND METHODS

Plant material

Two seed lots of black locust were tested. The first one was from Serres, East Macedonia, Greece (a local seed source of unknown provenance) with seeds collected in 2004 and provided by the Ministry of Rural Development & Food (Section of Forest Nurseries & Seed Production, Athens). The same seed source is used by the Forest Service nurseries (Greek origin). The second one (year of collection: 2006) was of Hungarian origin and came from a small seed orchard which was established near Thessaloniki in Central Macedonia, Greece (40°46'09"N, 22°21'03"E, altitude 10 m a.s.l.). A mass of 1,000 seeds for the two seed entries weighed 21.03 g (47.551 seeds·kg^{-1}) and 19.30 g (51.813 seeds·kg^{-1}), respectively (Spyroglou et al. 2009).

Pre-cultivation in mini-plugs

An experiment was carried out at the Forest Research Institute (National Agricultural Research Foundation – NAGREF) in Loutra Thermis, about 20 km SE of Thessaloniki, northern Greece.

Seed entries of Greek and Hungarian origin were tested in this experiment. Before sowing, the seeds were mechanically scarified for 75 min in a handmade cylindrical scarifier. After pretreatment, their germinability was found to be 86% and 81% for the Greek and Hungarian origin, respectively.

Two growing media were used: enriched peat (PE) (Klassmann TS1, Klassmann-Deilmann GmbH, Geeste, Germany, pH 6.0) and stabilized medium (PF) (Preforma PP01, Jiffy Products International AS, Stange, Norway, pH 4.0). The container trays filled with enriched peat were saturated with water to field capacity before sowing. The containers with stabilized medium were hydrated at source by the manufacturer. Mini-plug plastic trays were used, which were originally designed for pre-cultivation of horticultural crops (QuickPot®, Herkuplast-Kubern GmbH, Ering am Inn, Germany). The dimensions of all trays were 310 mm × 530 mm but the trays had four cavity densities, namely 3,500·m^{-2} (very high), 1,820·m^{-2} (high), 1,460·m^{-2} (medium) and 975·m^{-2} (low) with the respective cell volumes of 3, 9, 13 and 18 cm^3. One seed was placed into the substrate of each cell to a depth not more than three times the seed diameter (Hartmann, Kester 1983).

A completely randomized factorial design with three factors (seed origin, growing medium and mini-plug density) was employed with five replications resulting in 3,020 established seedlings.

After sowing, the mini-plug containers were placed into growth chambers of 400 l (KB8000FL, Termaks AS, Bergen, Norway) for a period of four weeks. Environmental conditions in the chambers were set as follows: 14 h photoperiod, 250 μmol·m^{-2} per s photosynthetic photon flux density (PPFD), 80 ± 10% relative humidity (RH) and 20/15°C light/dark temperature. Watering was applied every other day. The number of emerged seedlings was recorded on the 7th day after sowing and thereafter at weekly intervals until the 28th day (end of the experiment) for the plants in the growth cabinet. Percentage survival was based on the total number of cells sown. On the 28th day, 15 seedlings per treatment combination or 240 seedlings in total were randomly selected. The following growth parameters were measured: root length (RL), shoot height (SH), leaf area (including the shoot) (LA), root dry mass (RDM) and shoot dry mass (SDM). RL and SH were measured as the distance from the root collar to the lower and upper end of a seedling, respectively; LA was measured with an area meter (AM100, ADC Bioscientific Ltd., Herts, UK); and RDM and SDM with an analytical balance (E42S-B, Gibertini) after oven drying at 70°C for 48 h. The root/shoot ratio was also calculated on a dry mass basis (RDM/SDM). The ratio of variable to maximal fluorescence (F_v/F_m) (Maxwell, Johnson 2000) was measured in five seedlings per treatment combination (seed origin × growing media × mini-plug density) resulting in the evaluation of 80 seedlings. The effective quantum yield of photosystem II ($\Delta F/F'_m$) (Maxwell, Johnson 2000) was measured in 15 seedlings per treatment combination or 240 seedlings in total. Seedlings used for F_v/F_m were previously dark adapted for 20 min at least and measurements were done on the first round leaf in the 28th day using a pulse amplitude modulated photosynthesis yield analyzer (Mini-PAM, Heinz Walz GmbH, Effeltrich, Germany). In addition, chlorophyll content index (CCI), which derives from the ratio of optical absorbance at 655 nm to that at 940 nm and is unitless, was measured with a chlorophyll content meter (CCM-200, Opti-Sciences Inc., Hudson NH, USA) using a sample of 15 seedlings per treatment combination or 240 seedlings in total.

Root Growth Potential (RGP)

At the end of the cultivation period in growth chambers, 16 seedlings per treatment combination (seed origin × growing media × mini-plug density) were randomly selected. In total, 256 seedlings were used for the RGP test (16 seedlings × 2 seed origins × 2 growing media × 4 mini-plug densities). The selected seedlings were transplanted into mini-plug containers of each density. These containers were placed on top of stainless boxes (35 cm × 26 cm × 8 cm) filled with equal volumes of peat (Klassmann Base Substrate 250I, Klassmann-Delmann GmbH, Geeste, Germany) and sand. The boxes were immersed in a water bath, according to the standardized Root Growth Potential (RGP) technique described by Mattsson (1986), in a completely randomized experimental design containing four replications of four plants in each factor combination. During the 21 day test, water and air temperatures were maintained at 21 ± 2°C, relative humidity at 40 ± 10% and the photoperiod was set to 14 hours, with PPFD of 300 μmol·m^{-2}·s^{-1} at the plant level. Seedlings were watered every second day to maintain the growing medium water content at field capacity. Excess water was suctioned from each RGP box one hour after watering. No fertilizer was added during the test period. On the 21st day, all roots that had developed outside the mini-plug containers were washed in order to remove peat and sand, and cut off. RGP was assessed as the root dry mass (in mg) after oven drying at 70°C for 48 hours (Brønnum 2005).

Transplanting of mini-plugs to destination containers at the nursery

In May, trays with pre-cultivated seedlings were transported to the Dytikomakedonika Fytoria nursery in Grevena, western Macedonia (40°05'02"N, 21°25'53"E, altitude 520 m a.s.l.). The climate of the area is sub-humid Mediterranean with cold winters (mean annual rainfall 748.6 mm, mean maximum air temperature 25.4°C and mean minimum air temperature –0.9°C). Among the remaining (after the evaluation process) seedlings in the mini-plugs, 96 seedlings per treatment combination (2 seed origins × 2 growing media × 4 mini-plug densities) were randomly selected and transplanted in four replications of 24 seedlings each into destination containers. These are standard containers used in operational practice in Greece (QuickPot®, Herkuplast-Kubern GmbH, Ering am Inn, Germany), consisting of 240 cells·m^{-2} each of 330 cm^3 volume and 160 mm depth, filled with a mixture of loam, peat and perlite at a ratio of 5:3.5:1.5 (v/v/v), with pH 7.5 and watered before and after transplanting. Totally, 1,536 seedlings were transplanted. Plants were grown outdoors under a shade net (40% transparency), while the watering of seedlings was applied by sprinklers. No fertilization was applied.

Seedling survival was recorded at the end of the second cultivation period (after 24 weeks) in destination containers. In addition, growth and physiological measurements were taken using a random sample of five seedlings per seed origin, growing medium and density. Totally, 80 seedlings were evaluated.

Statistical analysis

Data was checked for normality of errors by plotting residuals versus predicted values, as well as by using the Kolmogorov-Smirnoff test for goodness of fit. Variances of data were tested for homogeneity using Bartlett's and Levenne's tests. All data was subjected to analysis of variance (ANOVA). Means of the four mini-plug density levels were further compared by Duncan's multiple range test at the 0.05 probability level when ANOVA produced significant results. Survival data did not satisfy the assumptions of ANOVA and they were subjected to the arcsine transformation for statistical analysis, but actual percentages are shown in the results. Pearson correlation was employed to examine the significance of the relationship between survival at the end of each cultivation phase and the physiological and growth responses also using the SPSS software (SPSS for Windows, Rel. 15.0. 2006, Chicago, SPSS Inc.).

RESULTS

Seedlings pre-cultivated in the growth chamber

The mean values of the parameters measured for the main treatments and their first order interactions are shown in Table 1. Several of them were significant and are described below (higher order interactions are not presented because they were not significant).

Germination and survival

Seed germination (germination energy) for all treatments ranged from less than 5% to more than 65% on the 7th day after sowing. Since that date the seedling germination increased and peaked on the 21st day to become unchanged until the end of the pre-cultivation period (four weeks). On the 28th day, the survival was significantly higher (*i*) in Hungarian than in Greek plant material by about 23%; (*ii*) in the growing medium of stabilized (PF) than in the nutrient-enriched (PE) peat by about 35%; (*iii*) in the very high than in the low mini-plug density by about 21%, while medium and high densities were intermediate and not significantly different from each other. Moreover, mini-plug density produced statistically significant interactions with both seed origin and growth medium.

Growth traits

Seedlings grown in PF had significantly longer roots by 24% than those in PE; and the high mini-plug density produced the longest roots, significantly different from the medium density and especially from the very high plug density. Also, the interaction between seed origin and growing medium was significant, as well as, between seed origin and mini-plug density.

Shoot height did not significantly differ either between the two plant sources or among the four cultivation densities. On the contrary, the growing medium produced significant differences with the SH of seedlings grown in stabilized medium being superior to that of seedlings grown in enriched peat by about 12% while its interaction with the other two factors was insignificant.

Leaf area was larger by 37% in stabilized than in nutrient-enriched growing medium. As far as the interactions are concerned, only growing medium × mini-plug density produced significant results.

RDM differed significantly only between the two growing media and among the four densities. Moreover, the growth medium produced significant interactions with both the seed origin and mini-plug density.

SDM was significantly higher by about 44% in seedlings grown in stabilized medium compared to those grown in enriched peat. The other factors did not produce any significant results.

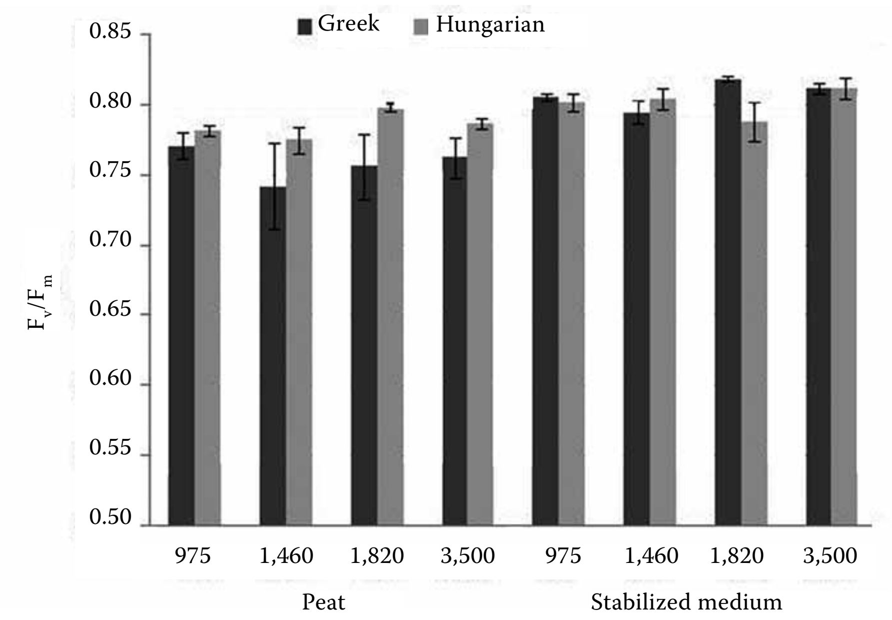
Finally, no significant results were produced by any of the three factors tested for RDM/SDM ratio. Only the interaction between seed origin and growing medium was found significant.

RGP was affected only by mini-plug density; it decreased with increasing cultivation density. However, the interaction of the three factors produced significant results.

Table 1. Mean values of survival, growth traits and root growth potential (RGP) of black locust seedlings after pre-cultivation in a growth chamber for 28 days for the three treatments (seed origin, growing medium and mini-plug density) and their first order interactions

Treatment	Survival (%)	Growth traits						RGP (mg)
		RL (cm)	SH (cm)	LA (cm^2)	RDM (mg)	SDM (mg)	RDM/SDM	
	***							
Greek (G)	51.43	5.73	8.02	8.09	5.27	17.23	0.334	24.72
Hungarian (H)	63.15	5.52	8.33	7.93	5.67	16.66	0.346	23.22
	***	***	***	***	***	***		
Enriched peat (PE)	48.68	5.02	7.71	6.77	4.65	13.88	0.371	24.87
Stabilized medium (PF)	65.90	6.23	8.63	9.25	6.29	20.01	0.310	23.08
	***	***			*			***
Low (l)	51.88c	6.02ab	8.33	8.29	5.79a	16.91	0.361	30.01a
Medium (m)	58.75ab	5.58b	8.00	8.01	5.72a	17.21	0.347	24.53b
High (h)	55.90bc	6.42a	8.48	8.24	5.75a	17.77	0.346	24.50b
Very high (vh)	62.64a	4.49c	7.88	7.48	4.63b	15.89	0.307	16.88c
		**			**		**	**
G*PE	41.58	5.39	7.58	6.74	4.90	13.84	0.393	23.25
G*PF	61.28	6.06	8.46	9.44	5.65	20.63	0.275	26.20
H*PE	55.78	4.64	7.85	6.80	4.41	13.91	0.348	26.49
H*PF	70.53	6.40	8.81	9.05	6.93	19.40	0.344	19.95
	**							*
G*l	41.25	5.88	8.11	8.17	4.81	16.33	0.328	31.62
G*m	55.83	5.86	8.18	8.10	5.92	17.82	0.357	23.23
G*h	49.74	6.79	8.19	8.67	5.71	18.34	0.352	28.48
G*vh	58.89	4.39	7.60	7.41	4.65	16.44	0.301	15.56
H*l	62.50	6.17	8.56	8.42	6.77	17.49	0.394	28.39
H*m	61.67	5.30	7.83	7.92	5.51	16.59	0.337	25.82
H*h	62.05	6.05	8.77	7.81	5.78	17.20	0.339	20.52
H*vh	66.39	4.59	8.16	7.55	4.62	15.35	0.314	18.16
	**	**		*	*			***
PE*l	46.75	5.25	7.99	7.18	4.76	13.94	0.381	35.79
PE*m	45.00	5.25	7.53	6.31	4.53	13.27	0.375	26.23
PE*h	47.69	5.24	7.61	6.49	4.62	13.72	0.377	21.18
PE*vh	55.28	4.33	7.72	7.09	4.71	14.56	0.349	16.28
PF*l	57.00	6.79	8.68	9.41	6.81	19.88	0.341	24.22
PF*m	72.50	5.90	8.47	9.71	6.90	21.14	0.318	22.83
PF*h	64.10	7.60	9.35	9.99	6.88	21.81	0.314	27.81
PF*vh	70.00	4.65	8.04	7.87	4.56	17.23	0.265	17.44

Growth traits: RL – root length, SH – shoot height, LA – leaf area, RDM – root dry mass, SDM – shoot dry mass, RDM/SDM – root dry mass to shoot dry mass ratio, RGP – root growth potential; Density levels: Low – 975 mini-plugs·m^{-2}, Medium – 1,460 mini-plugs·m^{-2}, High – 1,820 mini-plugs·m^{-2} and Very high – 3,500 mini-plugs·m^{-2}; ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$ (according to ANOVA); means of the mini-plug density treatments with different letters within the same column differ significantly at $P < 0.05$ (according to Duncan's test)



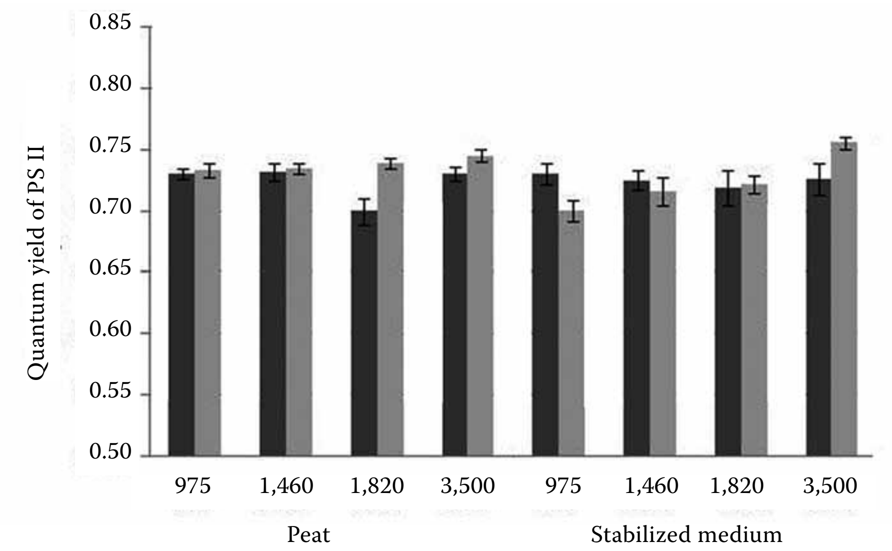


Fig. 1. The ratio of variable to maximal fluorescence F_v/F_m (top figure) and effective quantum yield of photosystem II (PS II) (bottom figure) of black locust seedlings of Greek and Hungarian origin, which were pre-cultivated for four weeks in mini-plugs of four densities (975; 1,460; 1,820 and 3,500 mini-plugs·m^{-2}) and two growing media (peat and stabilized medium). Vertical bars represent standard errors of the means

Chlorophyll fluorescence

F_v/F_m was found to be significantly affected only by the type of growing medium and the interaction between seed origin and growing medium. In both seed origins, the highest F_v/F_m values were observed in plants grown in stabilized medium (Fig. 1). No significant differences in $\Delta F/F'_m$ were found either between the seed origins or between the growing media types. However, mini-plug density significantly affected ($P < 0.05$) the $\Delta F/F'_m$, with the highest values being found in the plants grown at the very high density. In the Hungarian seed origin, growing medium, density and their interaction affected the quantum yield of seedlings. Seedlings of this origin pre-cultivated in stabilized medium and at the highest density had the highest quantum yield (Fig. 1).

Mini-plug seedlings transplanted into destination containers at the nursery

The results are reported in Table 2, which shows the mean values of the parameters measured for the three treatments and their first order interactions (higher order interactions are not shown because they were not statistically significant).

Survival

Mean seedling survival at the end of the first growing period in destination containers did not differ significantly between seed origins and among the four mini-plug densities; only growing medium produced significant results with the survival of

Table 2. Mean values of survival and growth traits for seed origin, growing medium and mini-plug density of black locust seedlings at the end of the growing season after transplanting into larger pots in a nursery

Treatment	Survival (%)	RL (cm)	SH (cm)	RDM (mg)	SDM (mg)	RDM/SDM
		*	***	**	**	
Greek (G)	53.66	18.26	29.16	1,335.36	1,686.93	1.055
Hungarian (H)	61.16	20.59	34.61	1,889.53	2,458.84	1.085
	***		***		***	**
Enriched peat (PE)	36.59	19.45	29.41	1,681.91	2,711.88	0.986
Stabilized medium (PF)	78.23	19.22	34.35	1,493.47	1,287.47	1.165
		*	***	*	***	
Low (l)	59.64	18.71b	31.25b	1,367.92b	1,848.77b	1.049
Medium (m)	57.29	19.01b	33.61ab	1,652.86ab	2,140.00ab	1.097
High (h)	57.09	20.92a	35.08a	1,902.21a	2,304.30a	1.078
Very high (vh)	55.61	18.55b	27.58c	1,405.86b	1,845.02b	1.048
		*			***	
G*PE	32.45	17.87	27.23	1,415.32	2,313.38	0.925
G*PF	74.86	18.65	31.09	1,255.40	1,060.49	1.186
H*PE	40.72	21.04	31.59	1,948.49	3,110.39	1.046
H*PF	81.59	19.99	37.62	1,810.91	1,590.11	1.137
	*		*		***	***
G*l	67.24a	16.90	28.20b	1,153.46	819.58c	1.406a
G*m	49.46ab	17.48	28.03b	1,184.41	897.05c	1.331a
G*h	41.95b	20.23	34.03a	1,880.72	2,869.50a	0.869b
G*vh	55.98ab	18.42	26.36b	1,122.85	2,161.60b	0.615b
H*l	52.04bc	20.53	34.30a	1,582.39	2,877.96a	0.691b
H*m	65.13ab	20.53	39.19a	2,121.31	3,382.95a	0.863b
H*h	72.22a	21.60	36.13a	1,923.70	1,739.10b	1.286b
H*vh	55.23c	18.80	28.80b	1,971.88	1,211.86b	1.914a
				**	**	
PE*l	41.36	18.40	30.55	1,712.95	2,760.57	0.970
PE*m	27.49	19.66	29.03	1,462.85	2,842.03	0.861
PE*h	40.70	20.75	32.05	1,847.54	3,000.21	0.931
PE*vh	36.80	19.00	26.00	1,704.28	2,244.71	1.180
PF*l	77.92	19.03	32.95	1,022.90b	936.97b	1.127
PF*m	87.10	18.35	38.19	1,842.87a	1,437.97a	1.334
PF*h	73.47	21.08	38.11	1,956.88a	1,608.39a	1.224
PF*vh	74.42	17.64	28.16	809.02b	1,045.64b	0.783

Growth traits: RL – root length, SH – shoot height, RDM – root dry mass, SDM – shoot dry mass, RDM/SDM – root dry mass to shoot dry mass ratio; Density levels: Low – 975 mini-plugs·m^{-2}; Medium – 1,460 mini-plugs·m^{-2}; High – 1,820 mini-plugs·m^{-2} and Very high – 3,500 mini-plugs·m^{-2}; ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$ (according to ANOVA); means of the mini-plug density treatments with different letters within the same column differ significantly at $P < 0.05$ (according to Duncan's test)

Table 3. Pearson correlation coefficients of physiological parameters and growth traits with survival of black locust seedlings at the end of pre-cultivation period in mini-plugs in growth chambers (after four weeks) and after transplanting to destination containers in a nursery (after 28 weeks)

Parameters	Survival	
	4 weeks	28 weeks
Survival (4 weeks)	–	0.496**
Physiological parameters (4 weeks)		
F_v/F_m	0.453**	ns
Yield	ns	ns
Growth traits (4 weeks)		
RL	ns	0.338**
SH	0.367**	0.349**
LA	0.382**	0.436**
RDM	0.372**	0.407**
SDM	0.451**	0.540**
RDM/SDM	ns	ns
Growth traits (28 weeks)		
RL	ns	ns
SH	0.449**	0.411**
RDM	ns	ns
SDM	ns	–0.376**
RDM/SDM	ns	ns

RL – root length, SH – shoot height, LA – leaf area, RDM – root dry mass, SDM – shoot dry mass, RDM/SDM – root dry mass to shoot dry mass ratio; n = 80 for the 4 weeks and n = 64 for the 28 weeks measurements
**P < 0.01, ns – not significant

seedlings pre-cultivated in stabilized medium exceeding by 117% that of seedlings grown in peat. Also, only the interaction between growing medium and density was significant.

Growth traits

Root length was longer by 13% in the seedlings of the Hungarian origin than in those of the Greek provenance. Seedlings grown at the higher densities had significantly longer roots than those grown at lower densities. As for interactions, only the seed origin produced significant results with density.

Shoot height was significantly affected by all three factors: seedlings of the Hungarian origin exceeded those of the Greek origin by 19%; seedlings grown in the stabilized medium exceeded those grown in peat by 17%; and seedlings in mini-plugs of high density exceeded those grown at the low and very high density by 12% and 29%, respectively. However, only the interaction between seed origin and density produced significant results.

Root dry mass differed significantly between the two seed sources with the Hungarian origin exceeding the Greek one by 41%; and among the four densities with the high density exceeding the low and the very high ones. As for interactions, significant effects were found only between growing medium and density.

Shoot dry mass was significantly affected by all three factors with the Hungarian origin exceeding the Greek one by 46%, the peat exceeding the stabilized medium by 111% and the high density exceeding the low and the very high ones. As far as the interactions are concerned, significant results were found between all three factors. Finally, in the case of the RDM/SDM ratio, only the growing medium produced significantly different results with seedlings grown in stabilized medium exceeding those in peat by 18%. As for interactions, only the seed origin significantly interacted with density.

Chlorophyll fluorescence and chlorophyll content

After transplanting to destination containers in the nursery all cultivation-derived differences in F_v/F_m or quantum yield disappeared (data not shown). Chlorophyll content was higher in seedlings of the Greek origin and when peat was used.

Correlations

The survival of seedlings after four weeks of pre-cultivation was significantly correlated with their final survival after another 24 weeks in destination containers in the nursery (Table 3). Of the two physiological variables F_v/F_m and effective quantum yield, only F_v/F_m was correlated with seedling survival after four weeks (Table 3). Statistically significant correlation coefficients between growth traits after four weeks and survival ranged from 0.367 to 0.451 and from 0.338 to 0.540 after 28 weeks (end of the first growing period). Of the growth traits recorded at the end of the first growing season (after 28 weeks of cultivation) only the shoot traits were correlated significantly with survival (Table 3).

DISCUSSION

Seed origin, growing medium and mini-plug density affected survival, germination and growth of black locust seedlings, but their relative contribution was different. Growing medium was much more important during the first stages of seedling growth in contrast to the seed origin which had more pronounced effects in later stages, while mini-plug density played a significant role throughout the entire experimental period.

Hungarian material had higher survival during the pre-cultivation period in the growth chamber and it was superior in all growth traits during the nursery period. This suggests its higher genetic potential compared to the Greek origin. As a matter of fact, Hungarian seed was collected from trees and stands that showed potential genetic superiority. Progenies derived from such excellent stands of trees generally outperform unselected stock. On the contrary, Greek seed was not collected from outstanding phenotypes. The genetic superiority of Hungarian seeds seems to have overcome their disadvantage of being relatively smaller than the Greek seeds. According to Baskin and Baskin (1998), small seeds are handicapped in germination as compared to larger ones affecting accordingly seedling survival and vigour characteristics. Such a disadvantage has not been confirmed in our experiment.

Growing medium was critical for the survival of seedlings in both periods of cultivation, but significant differences between growing medium types in relation to growth traits were observed only during the first phase. Seedlings grown in the stabilized medium consistently had a significantly better performance than those in enriched peat in all traits except the RDM/SDM ratio. Seedling performance was improved despite the fact that the pH of the stabilized medium was much lower (pH = 4.0) than that of peat and the suggested range of soil pH for black locust cultivation is 5.5–7.5 (Bridgen 1992). The superiority of stabilized medium to peat could be attributed to its more suitable physical and chemical properties (e.g. porosity, water retention etc.), which may have had an important impact on seedling development during the first phase of the experiment in growth chambers. During this phase, no differences in the root to shoot ratio were found between seedlings growing on the two different media substrates. In general, the root system of seedlings should be in balance with the shoots for good survival and early growth. The root/shoot ratio of about 1 is generally accepted as a good planting stock quality standard (Kennedy 1988). However, in the case of stabilized medium the low ratio seemed not to be critical for the extraction of mini-plugs from their containers before a firm root plug had been formed. Actually this is one of the system primary advantages, resulting in higher survival percentages after transplanting. Furthermore, the roots in a stabilized medium do not develop any typical deformities that often lead to structural defects in the transplants.

The positive influence of stabilized medium on plant performance, however, was not maintained after transplanting into destination containers although it was reported that in some cases this influence continued during the early period after planting (Costa et al. 2004). Nevertheless, mini-plug transplants grown in stabilized medium showed a significantly more favourable RDM/SDM ratio compared to those grown in peat, suggesting a better potential for survival and growth in the field (Mattsson 1996; Villar-Salvador et al. 2008). Improved seedling survival and growth traits of all seedlings grown in stabilized medium, particularly in the pre-cultivation stage, suggest that this substrate can enhance the performance of even relatively poor genetic material.

Mini-plug density played a significant role in seedling survival during the first phase of the experiment. After transplanting to destination containers in the nursery, the original mini-plug density did not have an impact on seedling survival.

During the pre-cultivation phase RL and RDM increased as density was decreased. According to Barnett (1980), there is a higher seedling competition for light at higher densities, which results in fewer carbohydrates available for root elongation and growth. The seedlings seem to have invested more in the development of their root systems, as shown by the RL and RDM values, when grown at low densities. Their aboveground parts did not seem to be significantly affected by the mini-plug densities used. This could probably be due to the short culture period (four weeks) in the growth chambers. It was previously reported that the effect of density depends on the length of the growing season (Barnett, Brissette 1986) and when longer seedling culture periods are used, density becomes more important in this regard (Barnett 1980). Thus, in this early stage, their competition for light might be minimal and the plug volume may be the most crucial factor for development of new roots.

RGP is the most common indicator of the physiological quality of planting stock and subsequent field performance for conifers, and it is also widely used for seedling physiological assessment in hardwoods (Wilson, Jacobs 2006). RGP was found to be significantly correlated with one-year height of ash (*Fraxinus excelsior* [L.]) (O'Reilly et al. 2002a) and with field growth and survival of two-year-old holm oak (*Quercus ilex* [L.]) (Pardos et al. 2003), while the low RGP at the time of planting was found to be related to reduced field survival and stem die back in two oak species (Garriou et al. 2000). In our experiment, RGP did not differ significantly between the two seed origins. The same results were reported for *Retama sphaerocarpa*, also a leguminous woody species (Villar-Salvador et al. 2008). In another RGP test, black locust developed a 5–10 times heavier root system in comparison with the conifer species tested (Kostopoulou et al. 2010). These results suggest that RGP is species-specific giving black locust a competitive advantage in forest plantations, especially in Mediterranean climate areas. The length of growing season in the Mediterranean climate is determined by the duration of low temperatures in the winter and by the summer drought (Mitrakos 1980). The rapid development of seedling root system before the onset of summer drought may provide an advantage for establishment on sites with long and dry summers.

RGP was not affected by the substrate but it decreased as mini-plug density increased suggesting that low densities provide more favourable conditions for the production of new roots. Similar findings have also been reported for *Pinus brutia* (Radoglou et al. 2011). This could be due to the increased seedling size associated with wider spacing, as it was also shown in *Pinus taeda* (South et al. 1990). Using lower density results in a lower competition of seedlings for light, which means that more carbohydrates can be used for the formation of new roots (Van den Driessche 1987).

Chlorophyll fluorescence tests have been used for quality testing of conifers and also of some hardwoods but the tests have limited applicability for deciduous hardwoods (Wilson, Jacobs 2006). Due to its correlation with seedling survival until the end of the pre-cultivation phase, the variable F_v/F_m could serve as a quality index. The ratio of F_v/F_m in dark-adapted leaves represents the efficiency of Photosystem II and is regarded as a sensitive index of plant photosynthetic yield (Maxwell, Johnson 2000). The values of F_v/F_m of the pre-cultivated material measured in our study were within the range (0.75–0.85) reported for healthy, unstressed plants (Maxwell, Johnson 2000).

After seedling transplanting to destination containers all differences in chlorophyll fluorescence variables observed by the end of the pre-cultivation phase disappeared. The higher chlorophyll content found in seedlings of the Greek origin could be attributed to the fact that the measurement was performed late in the autumn of 2007 and seedlings of the Hungarian origin initiated the phase of leaf abscission, which may have resulted in lower values of chlorophyll content by the time of measurement.

Because survival was significantly correlated with growth traits during the pre-cultivation phase, this period is crucial for seedling establishment after transplanting. Following transplanting only SH significantly affected survival. Taking into account the fact that SH is an easily obtained and non-destructive measure, this parameter could be used for the grading of black locust seedlings for transplanting. Mebrahtu (1992) reported that the total plant mass increased by the end of the second growing season with a large proportion of this dry matter being partitioned to branches in black locust. He also reported that the correlation between height and total dry mass was not always high due to the variation among plants in carbon partitioning to branches and roots. Since the height is usually used as a selection criterion in tree breeding, this is very significant because the selection for height might not result in trees with higher dry matter production.

Apart from seedling size and RDM/SDM ratio, the uniformity (small differences in plant size) of planting stock is an important issue, particularly if mechanical planting is to be used (O'Reilly et al. 2002b). With the employment of mini-plugs in planting stock production, the amount of seeds needed is much smaller than that needed for the production of bareroot seedlings. In addition, mini-plugs can be produced during the winter period and used early in the spring, thus ensuring planting stock availability early in the planting season. In addition, mini-plugs can be transferred to distant planting sites easily, safely and cost-effectively, something that cannot be accomplished with bareroot seedlings. According to Landis (2007), mini-plug transplants come closer to achieving nursery production goals such as: almost 100% yield, highest plant density per production area, maximum use efficiency of seeds, shorter crop rotation and improved stock quality (plants with large stem diameter and fibrous root systems). In general, while the cost of container-grown broadleaved plants may be three to five times higher than that of bareroot stock, higher survival rates in harsh sites can make the cost of container-grown seedlings very competitive (Missouri Department of Natural Resources 2006). On the other hand, studies which have included plug-transplant stock types or which have been set up to specifically compare bareroot vs. plug transplants have generally reported greater field performance of plug-transplants than in bareroot stock (Tanaka et al. 1988).

CONCLUSIONS

The growing medium is important at the precultivation phase of seedling growth with the stabilized type being superior to enriched peat in enhancing the performance of even relatively poor genetic material and increasing seedling survival after transplanting. The use of lower mini-plug densities resulted in better root growth while the medium to high densities enhanced shoot growth. The root growth potential was also favoured by low mini-plug densities.

Survival of seedlings was closely correlated with fluorescence and some growth traits measured at the end of the 28-day pre-cultivation phase, particularly with shoot height, suggesting that these parameters could be used for the grading of black locust seedlings for transplanting.

Due to their good performance and other benefits mini-plugs are recommended in the establishment of black locust plantations, particularly in harsh sites.

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