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CONTRIBUTION TO THE SELECTION OF PRODUCTIVE PROGENIES OF *ROBINIA PSEUDOACACIA* VAR. *MONOPHYLLA* CARR. FROM YOUNG PLANTATIONS IN NORTHERN GREECE

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ABSTRACT

Black locust (*Robinia pseudoacacia* L.) is a leguminous forest species, suitable for a variety of uses such as wood production of high quality (timber, poles, fuelwood), beekeeping, land reclamation and biomass production to be used either for energy or as forage. Thirteen clones of the *monophylla* variety of black locust (var. *monophylla* Carr. or *unifolia* Talou) were selected within the open pollinated families of a progeny test for their superior growth, their green foliage (in late autumn) and their small thorns (<15 mm). These clones were tested for two years in comparison with seven open pollinated families, within which they have been selected from, and seedlings coming from a mixture of seeds from all the open pollinated families of the progeny test representing the average population (C_0). Seedlings of a common black locust tree were also included in the test as a control. The comparison involved the length of the longest thorn, above ground biomass production (leaf, branch and total weight) and crude protein content of their leaves, aiming at selecting clones suitable for animal feeding. In clones, the longest thorn length and all the compounds of biomass produced statistically significant differences in both years while in families, leaf, branch and total weight were found non-significant during the second year. Oligophyllous type had significantly shorter length of the longest thorn and less leaf, branch and total weights than the polyphyllous type of clones in both years while in families the differences were non-significant between the two types for branch and total weight during both years. Clones had significantly higher crude protein content compared to the families. Overall, at least three polyphyllous clones had shorter longest thorn and were the most productive of all clones and families tested, compared to C_0 and the control in both years. The expected gains from selection of these clones were satisfactory.

Key words: *Robinia pseudoacacia* var. *monophylla*, selection, clones, half-sib families, thorn length, biomass, crude protein content

INTRODUCTION

Black locust (*Robinia pseudoacacia* L.) is a fast-growing, nitrogen-fixing leguminous tree species, native of south-eastern USA, but it has been extensively planted in many parts of USA for timber production and erosion control. It was the first forest tree species to be introduced from N. America to Europe and more specifically in France in 1601 for ornamental purposes. It soon became very popular in many European countries, but also in many other parts of the world. This wide spreading may be attributed to its adaptability to a large range of site conditions, potential to be easily improved, fast growth, high timber and honey yields, abundant and frequent seed crops, excellent rooting ability of the seedlings and the few pests and diseases that can damage it (KERESZTESI 1988; HANOVER 1990).

Black locust is a multi-purpose species suitable for lumber, poles, fuel, land reclamation, beekeeping, energy biomass, wood fiber and forage (BARRETT *et al.* 1990; BONGARTEN *et al.* 1992). For this latter use has attracted the attention as a potential perennial forage crop because of its ability to fix nitrogen and high protein content in stems and foliage (BARRETT 1992). KERESZTESI (1988) reports that black locust is widely used as fodder for animals and poultry (domestic and wild) in many regions of the world. In the Mediterranean region, for example, it was found to be a very important contributor to grazing animal nutrition in extensive livestock production systems (PAPANASTASIS *et al.* 1999; PAPACHRISTOU *et al.* 1999).

Although there is an increasing interest in the use of black locust as a forage plant, rather limited breeding work is available compared with the many cultivars for wood and honey production purposes that have been registered mainly in Hungary and other Central European countries. KERESZTESI (1992) has classified some Hungarian timber-producing cultivars as suitable for fodder production, too. Also, KERESZTESI (1988) reports that some cultivars for fodder production purposes have been developed in N. and S. Korea, mostly by mutation breeding, characterised by the absence of thorns or increased size of leaves and high protein content (colchitetraploid).

The main traits that an ideotype of black locust for fodder or forage production has to combine include high biomass production and regeneration ability, a high nutritive value and the absence or a reduced number and size of thorns. The pair of strong thorns is one of the main morphological characteristics of black locust. It has been assumed that they serve as mechanisms that prevent browsing by animals, attracted by its palatable and nutritious leaves and young shoots. However, this is doubtful because thorns do not provide much protection during the first year since they do not fully develop until the end of the growing season (MEBRAHTU & HANOVER 1989a). Anyway, thorns that arm the juvenile stems of black locust are generally very undesirable because they pose a hazard for human handlers, making tending and harvesting operations difficult (BONGARTEN 1992). As a consequence, farmers are very reluctant to use black locust as a fodder tree, although grazing animals have no problems (RODER 1992; DINI 1993). For this reason, selecting productive cultivars of black locust with high nutritive value and no or reduced thorns is of great economic interest.

Breeding for small thorns in black locust has the risk that rapid growth may be sacrificed because thorn length has been found to be positively correlated with juvenile growth (KENNEDY 1983; MEBRAHTU & HANOVER 1989A; DINI–PAPANASTASI & PANETSOS, 2000). An effort to surpass this problem could be through selection of the so called "correlation–breakers", i.e. segregating individuals within families which may receive unusual combinations of genes that improve both characteristics (LIBBY 1983).

Monophylla black locust (*Robinia pseudoacacia* var. *monophylla* Carr. or *unifolia* Talou) (KAVVADAS 1956; KERESZTESI 1983) is a botanical variety with a few leaflets per compound leaf and very promising for forage production (DINI-PAPANASTASI 1997). The half-sib progenies of this variety exhibit a great variation in the number of leaflets per compound leaf. Their number ranges from one (which is actually a single leaf) to 27 leaflets. The fewer the leaflets the larger is their size. In fact, variation is manifested not only between trees, but also within the same tree, with a tendency for the number of leaflets per compound leaf to increase from the bottom to the top of the tree (DINI-PAPANASTASI & PANETSOS 2000).

The objectives of this study were: (1) to investigate the possibility of selecting clones of *monophylla* black locust combining high biomass production, with high nutritive value and no or reduced thorns, for animal feeding purposes, (2) to compare these clones with the open-pollinated families, where they have derived from, so that the genetic gain of the selection is estimated.

MATERIALS AND METHODS

The experiment was carried out in the farm of the Forest Research Institute, 20 km NE of the city of Thessaloniki, northern Greece (40° 35' N and 22° 58' E). Soils of the study area are sandy loams, derived from deposits of the tertiary period, fairly deep (1–2 m) and alkaline (pH 7.7). Climate is semi-arid Mediterranean with 416 mm long-term mean annual rainfall, 31.7 °C mean maximum air temperature in July and 0.2 °C mean minimum in January (TSIONTSIS 1996).

The plant material tested included:

a) Ramets of clones, produced from rooted stem cuttings of:

(i) 12 outstanding individual trees belonging to 7 out of 14 open–pollinated tree families of *monophylla* black locust tested in a progeny test in the same farm in 1990 (DINI-PAPANASTASI & PANETSOS, 2000); these individuals were selected in November 1992 for their superior growth performance including their very dense, rich and green foliage, as compared to their neighbours (Table 1);

(ii) an interesting spontaneous tree of *monophylla* black locust found near the Strymon river in Serres, prefecture of Macedonia, Greece with very good growth, straight stem, rich foliage without thorns and of about 25 years of age (to be named hereafter «Strymon» clone);

(iii) The thornless and sterile globe locust (*R. pseudoacacia* var. *umbraculifera* D.C.), normally found as grafted tree on common black locust rootstock, that was propagated by stem cuttings (DINI-PAPANASTASI, 2000).

b) Seedlings of half-sib families produced from seeds of:

(i) the 7 out of 14 *monophylla* black locust families of the progeny test, where the 12 above mentioned clones (case a,i) belonged (Table 1);

(ii) a seed mixture, composed of 20 seeds from each one of all the 14 *monophylla* black locust families of the progeny test, representing the average population and symbolized as C_0; and

Table 1. Clones, their leaf morphology type and the corresponding family of *monophylla* black locust, in each group of clones, within they have been selected.

No	Clone	Leaf morphology type *	Family
1	A-8A(4)	II	8A
2	A-8A(7)	I	
3	A-8A(9)	I	
4	B-2B(8)	II	2B
5	C-2B(13)	I	
6	A-2B(15)	I	
7	A-A(7)	II	A
8	B-A(13)	I	
9	A-7A(6)	II	7B
10	A-B(3)	II	B
11	B-3A(7)	I	3A
12	B-6A(8)	II	6A
13	Strymon	I	
14	Globe locust		
			C_0
			C

* I – oligophyllous type and II – polyphyllous type.

(iii) a randomly chosen common black locust tree (*R. pseudoacacia* L.), growing in another experimental farm (not connected with the above mentioned material), as a control (C).

For the production of ramets, hardwood cuttings were collected in January 1993 and planted in rooting benches in the greenhouse in order to have them rooted (DINI & PANETSOS 1994). The rooted cuttings were transplanted to plastic bags. Due to the different rooting ability of the clones, unequal number of ramets per clone were finally produced.

For the production of seedlings, seeds were collected in 1992. In April 1993, they were sown in paper pots after being soaked in hot water for 24 hours to facilitate their germination and powdered with fungicide to protect them from diseases. Following their germination in a few months, they were transplanted in plastic bags, the same as the ones used for the cuttings. No particular problems were faced in the production of seedlings.

The clonal test was established in the field in April 1994. To accommodate the problem of unequal number of ramets available per clone, a completely randomized experimental design with unequal samples was employed (STEEL & TORRIE 1980). Altogether 420 saplings were planted in 30 rows with 14 individuals each in a spacing 2.5 × 2.5 m. Also, a row of one-year old barerooted common black locust seedlings was established around the plantation with the same spacing to serve as a buffer zone. Both ramets and seedlings were cut at the end of the first and the subsequent growing seasons.

Measurements were carried out at the end of the first growing season (end of October – beginning of November) in 1994 and repeated in 1995. The following characters were included in the measurements:

Leaf morphology: the saplings were classified into the oligophyllous (few and large leaflets per compound leaf – type I) and polyphyllous (many and small leaflets per compound leaf – type II) (DINI–PAPANASTASI & PANETSOS, 2000) in both years;

1. Length of the longest thorn (as an indicator of thorniness of the black locust);
2. Aboveground biomass: all saplings from each clone and 20 saplings randomly taken from each family were cut at 20 cm aboveground and weighed. Then a representative sub-sample from each of them was taken, weighed, separated into leaves and branches and weighed so that the fresh weight of each sampling is estimated. Subsequently, the separated leaves and branches from each sub-sample were transferred to the laboratory, where they were oven-dried at 65°C for 48 hours and weighed. The dry weight of the sub-samples were then used to calculate the corresponding dry weights of the whole samplings;
3. Crude protein content: From all the members of each clone and family, and from the oven-dried sub-samples that were used for the determination of dry weight, equal samples of compound leaves were taken and analyzed for nitrogen (N) content with the macro-Kjeldhal method (A.O.A.C. 1990); and crude protein (CP) on a dry matter basis (DM basis) was calculated as N × 6.25.

Statistical analyses included one way analysis of variance for each character among clones, among families and combined (among clones and families) (STEEL & TORRIE 1980). Subsequently, the clones and families were ranked for all the biomass data on the basis of the performance of the average population (C_0) plus one or two standard deviations ($C_0+1\sigma$ or $C_0+2\sigma$), while for the length of the longest thorn the ranking was done also on the basis of C_0 but minus 1σ and 2σ since the objective was to select for reduced length. The standard deviation was calculated from the one- way ANOVA table as follows:

$$\sigma = \sqrt{\frac{ErrorMS}{r}}$$

where: MS = Mean squares, r = mean number of saplings in the clones and families.

In addition, the t-test was applied to compare the number of saplings belonging to the oligophyllous and polyphyllous types within clones and families as well as their crude protein content.

For estimating the heritability of families (h_f^2) the following formula was applied:

$$h_f^2 = \frac{\sigma_f^2}{\sigma_f^2 + \sigma_e^2}$$

where: σ_f^2 = family component of variance, σ_e^2 = variance due to the trees within each family.

For clones, the same formula was used but σ_f^2 was replaced by σ_c^2 indicating the clone component of variance.

For the calculation of the upper and low confidence limits with probability 90 % of the heritability coefficients, the following formulas were used (KNAPP *et al.* 1985):

$$UCL = 1 - \{F_{exp.} * (F_{0.05})\}^{-1} \text{ and}$$
$$LCL = 1 - \{F_{exp.} * (F_{0.05})^{-1}\}^{-1}$$

where $F_{exp.}$= the F value of the ANOVA table, $F_{0 \cdot 05}$ = The F value of the statistical table with probability of $p \leq 0.05$.

Finally, the expected genetic gain were calculated by using the following formula (PANETSOS 1986):

$$G = S * h_c^2 = (\text{Mean of selected clones} - C_0) * h_c^2$$

Where h_c^2 = heritability of clones, S = selection differential, C_0 = mean population.

RESULTS AND DISCUSSION

Leaf morphology characters

All the ramets of the *monophylla* clones maintained the leaf morphology type (I – oligophyllous or II – polyphyllous) of their ortet trees as described by DINI-PAPANASTASI and PANETSOS (2000). On the contrary, both types of trees coexisted within all the half-sib families, as it was expected.

Length of the longest thorn

All the ramets derived from the globe locust were

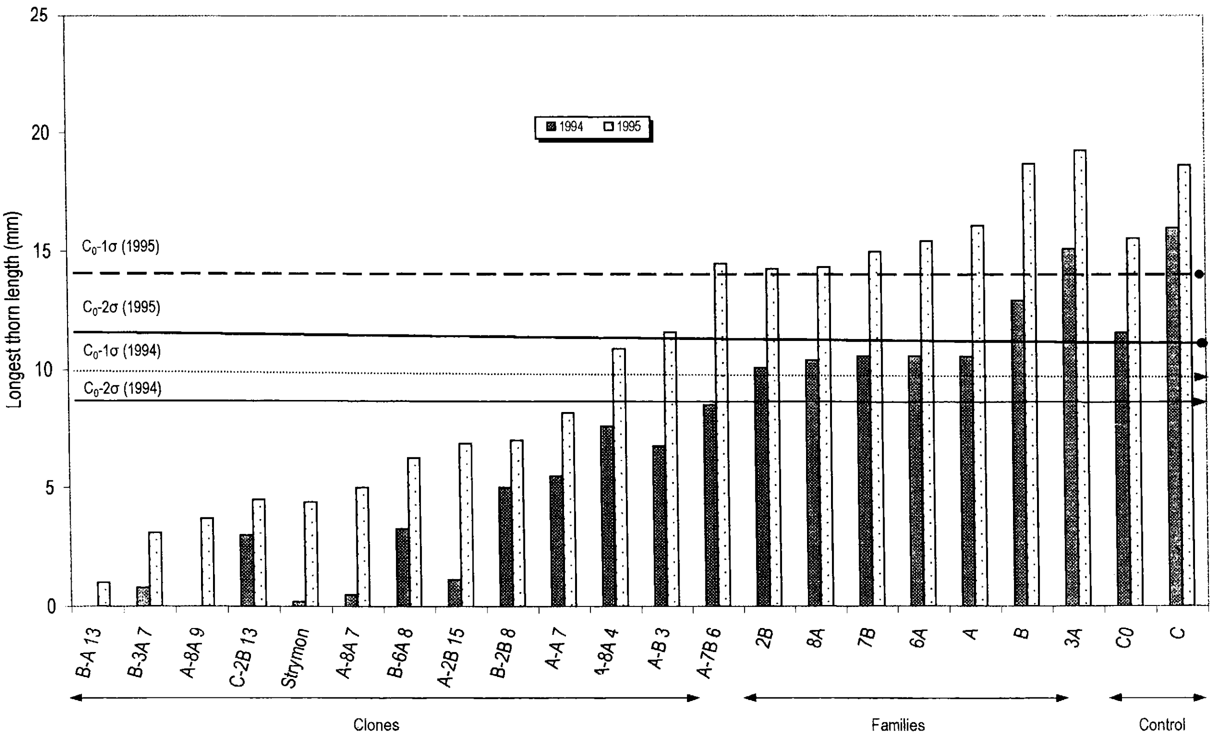

Figure 1. Ranking of clones and families according to the mean length of the longest thorn in 1994 and 1995 (in 1994 mean $C_0 - 1\sigma = 9.9$, mean $C_0 - 2\sigma = 8.2$; in 1995 mean $C_0 - 1\sigma = 13.5$, mean $C_0 - 2\sigma = 11.4$).

Table 2. One-way analyses of variance of various traits

Source	Year	TL			DLW			DBW			TPDW		
		df	MS		df	MS		df	MS		df	MS	
Clones	1994	12	85.61	**	12	22268.70	**	12	21905.20	*	12	86801.60	**
Error		80	3.62		79	6324.90		79	9887.40		79	29591.00	
Families		8	151.04	**	8	14570.60	*	8	55274.23	**	8	123314.80	**
Error		259	18.50		172	6593.50		173	20207.50		173	45084.40	
Combined		21	343.70	**	21	110673.77	**	21	18607.04	**	21	43989.95	**
Error		349	15.09		252	40277.32		251	6755.69		251	17000.47	
Colnes	1995	12	93.57	**	12	200006.90	**	12	398246.40	**	12	1584836.70	**
Error		81	10.90		79	32279.40		79	156411.10		79	332852.50	
Families		8	120.03	**	8	57420.60	ns	8	436463.40	ns	8	790276.40	ns
Error		269	28.29		173	38373.30		173	238641.90		173	450513.40	
Combined		21	389.42	**	21	136286.89	**	21	622819.94	**	21	1269624.39	**
Error		350	24.31		252	38657.40		252	212863.17		252	413627.60	

* $P < 0.05$, ** $P < 0.01$, and NS: not significant.

maintained completely thornless, just like their ortet tree, in both years of experimentation. This, however, did not happen in the *monophylla* black locust.

The mean length of the longest thorn for both clones and half-sib families is presented in Figure 1. In the first year, clones A-8A (9) and B-A (13) were completely thornless, while some of the ramets of the clones A-8A (7), A-2B(15), B-3A(7) and Strymon were also thornless. The half-sib families had less variation in the mean but higher in the individual values compared to clones. The longest thorn was produced by the control treatment (common black locust), which had a mean of 16 mm and a range from 8 to 28 mm. The statistical analysis produced significant differences both within clones and within families. The same was found in the combined analysis of both progenies (Table 2).

In 1995, mean length of thorns was increased by 104 % in the clones. The clones A-8A (9), B-A (13) produced thorns this year but both of them as well as B-3A (7) maintained certain ramets without any thorns. In the half-sib families, the average increase of the thorn was 38%. The control treatment had the highest range in individual values of the longest thorn length together with the *monophylla* family 3A but its mean length was shorter than the families 3A and B (Fig. 1). The statistical analysis produced significant differences both among clones and among families, while clones had again statistically shorter length of the longest thorn than the families (Table 2).

As far as the two leaf morphology types are concerned, oligophyllous type had significantly shorter thorn length than the polyphyllous in both years (Table 3). Also, although not measured, the polyphyllous type appeared to have more thorns than the oligophyllous one. It should be noted that the length of the longest thorn is a reliable and objective trait

Table 3. Means of individual traits of two types of clones or two types of individuals within families (oligophyllous – I and polyphyllous – II) with their t-test values for two years (1994–1995).

Progeny type	Traits	1994				1995			
		Type I	Type II	t-test value		Type I	Type II	t-test value	
CLones	Longest thorn length (mm)	0.5	6.6	13.32	***	4.1	10.1	8.14	***
	Leaf weight (g/plant)	84.5	152.8	3.76	***	200.1	427.7	4.99	***
	Branch weight (g/plant)	76.8	124.0	2.14	*	294.1	636.0	3.66	***
	Total weight (g/plant)	159.6	275.9	3.02	**	494.2	1063.8	4.21	***
Families	Longest thorn length (mm)	10.1	13.4	6.21	***	14.7	17.6	4.4	***
	Leaf weight (g/plant)	109.8	141.6	2.51	*	271.1	352.2	2.78	*
	Branch weight (g/plant)	141.2	173.3	1.45	ns	525.7	657.9	1.78	ns
	Total weight (g/plant)	250.9	313.4	1.9	ns	796.8	1010.1	2.1	*

but it does not seem to give a good picture of thorniness in black locust. Although they produced some thorns particularly in the lower parts, clones practically looked thornless since a large part of their foliage did not have any thorns, even in the second year. This means that the number of thorns should be also measured or estimated if a more reliable picture of the thorniness of black locust is to be desired (DINI-PAPANASTASI & PANETSOS, 2000). On the contrary, families appeared to have more thorns than clones.

Contrary to the number and size of leaves, which are inherited by the ramets of each clone, thorns seem to be affected mainly by other factors. According to SCHAFFALITZKY DE MUCKADELL (1959) mentioned by KENNEDY (1983), thorn is a juvenile character for common black locust, but it is also affected by shade. DINI– PA-PANASTASI & PANETSOS (2000) also reported for *monophylla* black locust that the oligophyllous type trees had fewer and smaller thorns than the ones of the polyphyllous type, which is associated with increased growth. In other words, thorn number and length is positively correlated with growth and with the number of leaflets per compound leaf.

The fact that the ramets of the same clone had variable sizes of thorns and co-existed with completely thornless ones should be attributed to the topophysis hypothesis. This hypothesis can be supported by the fact that among the 12 ortet trees of the original plantation, only A-8A(9) was practically thornless, while the others had simply very short thorns. This is because the stem cuttings were cut from the top of trees which has an advanced growth stage (PANETSOS 1986) thus producing shorter thorns (SCHAFFALITZKY DE MUCKADELL 1959 mentioned by KENNEDY 1983). The increase of the thorn length during the second year should be attributed to the cutting of trees at the end of first year, which suspended the maturity (LIBBY & HODD 1976) and rejuvenated the trees thus resulting in longer thorns.

It seems therefore that the production of completely thornless clones in *monophylla* black locust is rather unrealistic breeding endeavor. Nevertheless, several clones were produced with much shorter and much fewer thorns than the common black locust. It remains to be seen what could happen to the thorns with the absence of the annual clear cutting, which was applied in this experiment.

Biomass

Globe locust had a very different growth pattern compared with the *monophylla* and common black locust; it was characterized by a prostrate form with a very dense branch development and much smaller leaflets per compound leaf and its biomass production was limited. For this reason, it was excluded from the statistical analysis so that the variability estimations for clones are not influenced.

The mean values for the biomass traits of the *monophylla* black locust are shown in Figure 2. It is clear that leaf and branch weights had a wide variation in both clones and half-sib families and in both years. However, this variation was larger in clones than families and in the second than in the first year. As a result, the mean values of total weight (leaves and branches) of clones ranged from 80 to 532 g

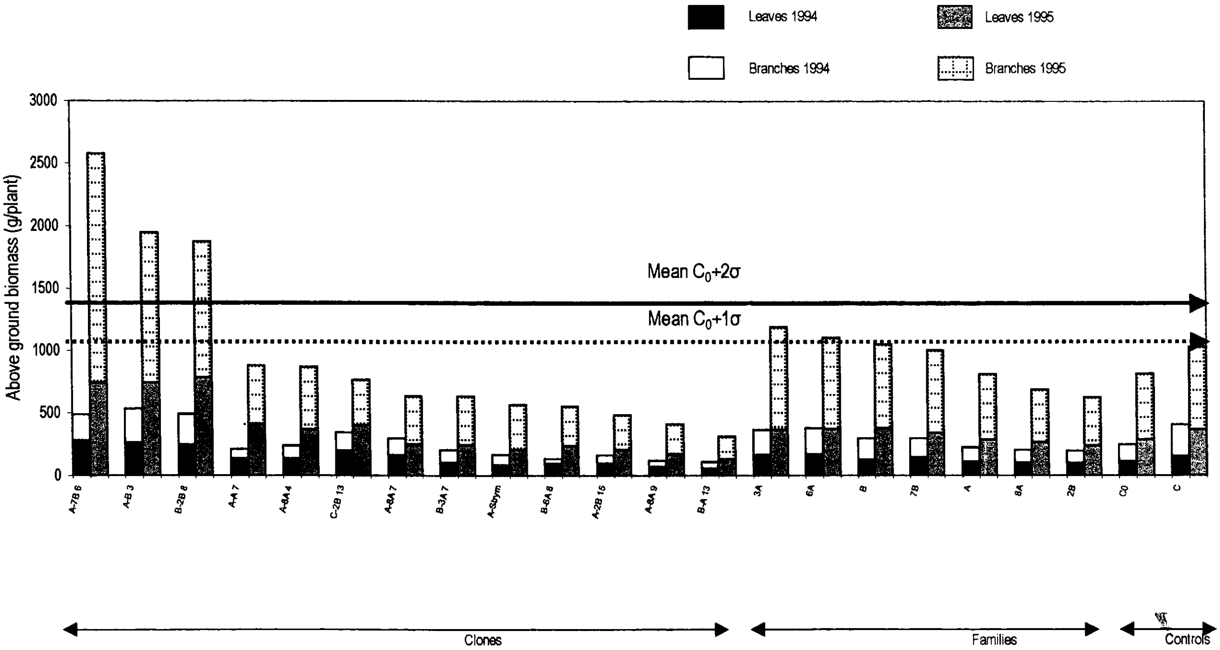

Figure 2. Ranking of clones and families according to their above ground biomass production (1994–1995) (in 1995 mean C_0 +1σ = 1088 and mean C_0 +2σ =**1362.**

plant^{-1} in 1994 and 310 to 2576 g plant^{-1} in 1995, an average increase of 254 %. For the half-sib families, the variation of the mean values in 1994 was from 197 to 406 and from 624 to 1191 g plant^{-1} in 1995, an average increase of 226 %. Overall, clones A-7B (6), A-B (3) and B-2B (8) were the most productive progenies, especially in the second year when far exceeded even the most productive families (Fig. 2).

The statistical analysis produced significant differences for all weight traits in both years for clones but only in the first year for families while the combined analysis of both progeny types produced significant differences in both years (Table 2).

The large variation observed in the weight parameters of both clones and families should be attributed to some extremely low values, which probably have been due the adverse microenvironment of the experimental area or to the difficulties in the initial establishment of the plants. Specifically for the clones, part of the variation within each of them may have been also caused by the differences in the ramets derived from rooting of stem cuttings. MEBRAHTU & HANOVER (1989b) have attributed similar large variation within clones of black locust to the different sizes of the root cuttings used for their production.

The difference in biomass between the first and the second year of the experiment reflect the stump sprouting capacity of the individual clones and families studied. The average increase of biomass in clones was higher (254 %) than in families (226 %). The individual values ranged from 114 to 430 % for clones and from 153 to 265 % in families. These percentages are much higher than the ones attained in other fast growing species such as the plain trees, where PANETSOS (1989) has found stump sprouting capacities 63.5 % for *Platanus occidentalis* and 56.4 % for *P. orientalis*. Of the three productive clones, A-7B (6) had the highest sprouting capacity followed by B–2B (8) and A-B (3). Among the families, no substantial differences were recorded but all of them had higher percentages than the control.

Polyphyllous clones produced significantly higher total, leaf and branch biomass in both years. Leaf biomass was significantly higher in the polyphyllous trees of the families, too but not the branch biomass, while total biomass was significantly higher only in the second year (Table 3). The fact that the polyphyllous clones were more productive compared with the oligophyllous ones confirms the results of the progeny test carried out earlier (DINI–PAPANASTASI & PANETSOS, 2000). In addition, the fact that polyphyllous clones had longer longest thorn than the oligophyllous ones is also supported by the positive correlation of growth (height and diameter) with increased thorn size, which was found in the same progeny test.

Ranking of clones and families

For ranking the 13 clones and 9 families, the population C_0 was used as a base. As far as the thorn length is concerned, all clones had mean values less than C_0 and the control by at least 2σ in 1994 (Figure 1). Among the most productive clones, B-2B (8) had shorter thorns than C_0 by 57 % and the control by 66 %, A-B (3) by 41 % and 58 % and A-7B (6) by 27 % and 47 % respectively for C_0 and the control. In 1995, clone B-2B (8) maintained the ranking order of the previous year (difference at least 2σ from both C_0 and control). In the other two clones, the differences were reduced but they were still maintained superior to C_0 and the control except A-7B (6), which did not significantly differ only from C_0.

For total weight, the first three cells of the ranking scale were occupied by the polyphyllous clones A-B (3), A-7B (6) and B-2B (8) which exceeded C_0 by more than 2σ in both years (Figure 2). More specifically, A-B (3) exceeded C_0 by 117 % and the control by 31 % in 1994 and by 139 % and 89 % respectively in 1995. Clone A-7B (6) exceeded C_0 by 98 % and the control by 20 % in 1994 while in 1995 the differences were 216 % and 150 % respectively for C_0 and the control. Finally, B-2B (8) exceeded C_0 and the control respectively by 100 % and 21 % in 1994 and by 130 % and 82 % in 1995. Among families, the most promising in terms of total weight were 3A and 6A (Figure 2). Similar results with the total weight appeared also when leaf and branch weight were considered separately (Figure 2). On the other hand, the control was also superior of C_0 by more than 1σ which indicates that common black locust is more productive than the average population of *monophylla* black locust, a result also found by PAPANASTASIS *et al.* (1998).

Crude protein content

Clones contained significantly higher crude protein (by 18 %) than families (Table 4). This difference should be attributed to the fact that the selection favored the late growing trees, which continued to have green leaves in autumn. On the contrary, no significant differences were found between the two leaf morphology types of clones (Table 4).

Heritabilities

During the first year of the experiment, the heritability estimates for the various characters studied ranged from 0.55 to 0.997 for clones and from 0.52 to 0.88 for families (Table 5). For the branch weight, the heritability estimate were higher in families than in clones, and for the total dry weight almost the same, although the opposite would be expected, due to the great amount of additive genetic variation which is captured by

Table 4. Comparison of mean crude protein content (DM basis, g/kg) of clones vs families and oligophyllous (type I) vs polyphyllous (type II) clones with their t-test values.)

Type of comparison	Clone	Families	t-test value
Clone vs. families	173.6	146.8	7.01***
	Clone type I	Clone type II	t-test value
Type I vs. type II	172.0	175.3	0.52 NS

*** P<0.001 and NS: not significant

Table 5. Heritability estimates with their upper and lower confidence limits (90 %) of clones and families for individual traits (1994 and 1995).

Trait	1994				1995			
	Clones		Families		Clones		Families	
	h^2	LCL–UCL *	h^2	LCL–UCL	h^2	LCL–UCL	h^2	LCL–UCL
Leaf weight (g/plant)	0.72	0.47–0.85	0.52	0.05–0.76	0.80	0.63–0.90	0.33	0.00–0.66
Branch weight (g/plant)	0.55	0.46–0.76	0.64	0.28–0.82	0.78	0.58–0.88	0.45	0.00–0.73
Total weight (g/plant)	0.66	0.36–0.36	0.63	0.26–0.81	0.79	0.60–0.89	0.43	0.00–0.71
Longest thorn length (mm)	0.997	0.92–0.98	0.88	0.76–0.94	0.88	0.78–0.94	0.76	0.55–0.88

Table 6. Expected genetic gains from the selection of the three best clones based on the high biomass production (g/plant) and the small thorn (mm) length compared with the mean population C_0 and the control (common black locust) during 1995 (in brackets the confidence limits of genetic gain for probability 90 %).

Trait	Compared with	Selected clones		
		A-B (3)	A-7B (6)	B-2B (8)
Longest thorn length	C_0	3.52 (3.12–3.76)	1.0 (0.7–1.0)	7.6 (6.7–8.1)
Leaf weight		365 (287–410)	396 (288–411)	398 (313–447)
Branch weight		528 (393–596)	1018 (757–1304)	440 (327–496)
Total weight		894 (679–1007)	1392 (1057–1568)	838 (637–944)
Longest thorn length	Common black locust (C)	6.2 (5.5–6.6)	3.6 (3.2–3.9)	10.2 (9.0–10.9)
Leaf weight		299 (236–337)	300 (236–338)	332 (261–374)
Branch weight		424 (316–479)	914 (680–1031)	336 (250–379)
Total weight		724 (550–816)	1222 (928–1377)	668 (508–753)

clonal selection (LIBBY 1983). These high family heritability estimates can be attributed to the large and equal number of seedlings per family and especially to the fact that a lot of vigorous plant seedlings of uniform size were available. This uniformity of plant stock increased the h^2 by reducing the phenotypic variance. In the case of clones, on the contrary, the lack of uniformity of propagules within each clone probably prevented a fast and uniform establishment of all the plants during the first year, with a negative impact on heritability estimates. The opposite occurred in the case of thorn length where clearly higher heritabilities were estimated for clones than for families with very narrow confidence limits.

In the second year of the experimentation the heritability estimates for the clones were higher than those for families (Table 5). For all the compounds of biomass, the heritability estimates for clones ranged from 0.78 to 0.80 while their confidence limits ranged from 0.58 to 0.90. The corresponding heritability estimates for the families were reduced and ranged from 0.33 to 0.45 while the confidence limits became wider with a great reduction of the lower one. The thorn length heritability estimates for the second year were lower for both clones and families with a reduction of 12% and 14% respectively with "widening" of the confidence limits to the lower, especially in the families (Table 5).

In general, the higher heritability estimates for the clones during the second year, in comparison with the families, could be attributed to their good establishment in the plantation and the consequent smoothing of environmental variance with the positive effect to heritability estimates increment (NAMKOONG & CONKLE 1976).

Expected genetic gain

Table 6 shows the expected genetic gains for the three clones A-B (3), A-7B (6) and B-2B (8) for the most important traits, namely leaf weight, branch weight, total biomass and thorn length, as compared to the mean population C_0 in 1995. It is clear that these gains were considerable, especially in total biomass production. Among the three clones with the best performance, B-2B (8) had the lowest biomass gains but the highest in reduced thorn length.

In Table 6, the expected genetic gains from the selection of the three promising clones, as compared to the control during 1995, are also presented. They were also remarkably high for the biomass production, although lower than the ones compared with C_0. Nevertheless, the expected genetic gains of the thorn length were much more higher compared to C_0.

The estimated heritabilities were most probably overestimated having consequently a positive impact on the corresponding values of the expected genetic gains. However, the above positive results for the three selected clones do not change even though we consider the lower confidence limits of the expected genetic gains. The results suggest that clones A-7B (6), A-B (3) and B-2B (8) are very promising for increased biomass production with a few thorns and high crude protein content in the leaves. Their superiority is maintained in the following the first stage years since the clonal test is still going on. However, more testing is needed before these three clones are released for commercial purposes.

Considering all the results mentioned above, it could be concluded that the selection of clones combining high biomass production, high regeneration ability after cutting, reduced thorn length and high CP content of the leaves is feasible. In addition, genetic gains achieved for the most important traits namely leaf weight, branch weight, total biomass and thorn length, compared with the mean population C_0, where the clones derived from, and the common *Robinia* (control) are considered satisfactory. In general, polyphyllous clones were more productive and with longer their longest thorn compared with the oligophyllous ones.

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