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Original article

Provenance variation in *Pinus nigra* at three sites in Northern Greece

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Abstract – Seventeen provenances of *Pinus nigra* Arnold from Greece, Turkey, Corsica and Calabria were tested on three sites in northern Greece. Data of the 9th year of tree height, diameter and survival show differences in growth on two sites with varying results per site, except for the Corsican provenance ranking high in both sites. Survival differences were identified only in one site with Calabrian (82%) and Corsican (87%) provenances ranking low. The analysis for twelve provenances common to the two sites where differences were identified, show significant site–provenance interactions. Some of the Greek provenances, especially the one from Thassos island grow better on the schist site with sandy loam soil, while the Turkish provenances grow better on the conglomerate site with clay loam soil. The existence of *Pinus nigra* ecotypes adapted to original site conditions is discussed.

***Pinus nigra* / provenance trials / site-provenance interactions / Greece**

Résumé – Variations entre provenances de *Pinus nigra* dans trois sites du Nord de la Grèce. Dix-sept provenances de *Pinus nigra* Arnold de Grèce, Turquie, France (Corse) et Italie (Calabre) ont été testées dans trois sites du nord de la Grèce. L'analyse des mesures de la neuvième année concernant la hauteur, le diamètre et le taux de survie, a montré des différences d'accroissement sur deux sites, avec des résultats variant dans chaque site, à l'exception de la provenance Corse dont l'accroissement était important sur les deux sites. Des différences de taux de survie ont été identifiées sur un site seulement où les provenances de Calabre (82 %) et de Corse (87 %) ont montré un taux relativement bas. Des interactions significatives entre provenances et sites ont été identifiées pour les douze provenances communes aux deux sites où des différences entre provenances ont été constatées. Quelques provenances grecques, particulièrement celle de Thassos, montrent un meilleur accroissement sur schiste et sol à texture sablo-limoneuse, tandis que les provenances turques donnent de meilleurs résultats sur conglomérat et sol à texture argilo-limoneuse. L'existence d'écotypes de *Pinus nigra* adaptés aux conditions d'origine est discutée.

***Pinus nigra* / essais de provenance / interactions provenances-sites / Grèce**

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1. INTRODUCTION

Industrial plantations of fast-growing conifers have been deemed necessary to Greece in order to meet future projections of national wood demand [10]. The aim of such plantations should be to meet future industry requirements by producing softwood timber of a size, uniformity and quality suitable for pulp, sawlogs and any other industrial use. Degraded oak coppice zones of eastern and western Macedonia are regarded as priority areas for the establishment of such plantations [15] due to their low current productivity, large extent, favourable site conditions and proximity to existing and potential wood markets and industries. *Pinus nigra* (Arnold), extensively used for reforestation in Greece, is considered as one of the most suitable conifer species for industrial plantations in that zone.

Pinus nigra (Arnold) is a southern European species with extended natural range, from about 5° west longitude in Spain and Morocco to some 40° east in the Turkish Anatolia. Its latitudinal range is also quite large, from 35° north latitude in Morocco and Cyprus, to 48° in north-eastern Austria and up to 45° in Crimea. The largest extensions are in eastern Spain, Balkans and western Turkey. Between these concentrations the distribution, on the northern mountains and on Mediterranean islands and peninsulas, is discontinuous and patchy [5]. This natural distribution pattern resulted in differentiation of populations in categories not fully agreed by the various authors. Thus Mirov [14] distinguishes two sub-species, namely Occidentalis (west of Italy) and Orientalis (east of Italy). Dallimore and Jackson [6] recognise four varieties i.e. nigra (Austria, north and central Italy, Greece and former Yugoslavia), caramanica (Crimea, Asia Minor and eastern Balkans), cevennensis (Cévennes of France, the Pyrénées, central and eastern Spain) and maritima (Corsica and southern Italy). Arbez and Millier [3] found that origins from Spain and south of France are similar, the Corsican one quite distinct, the Calabrian one transitional towards var. nigricans (Austria) and the Turkish, Bulgarian and Crimean origins generally similar but distinct from the western origins. Roman-Amat and Arbez [20] underlined the difficulty of finding a neat limit for spps nigricans and pallasiana as they appear to intermix freely at places, particularly in Bulgaria. Bassiotis [4] concluded that the Greek black pine populations, although similar to var. austriaca in some cases and to pallasiana (Asia Minor) in others, were distinct from other races of the species.

All these studies based mostly on needle morphology indicate the difficulties to differentiate black pine varieties on a regional basis. Wheeler et al. [29] commenting on provenance trials in USA stated that the 13 seedlots

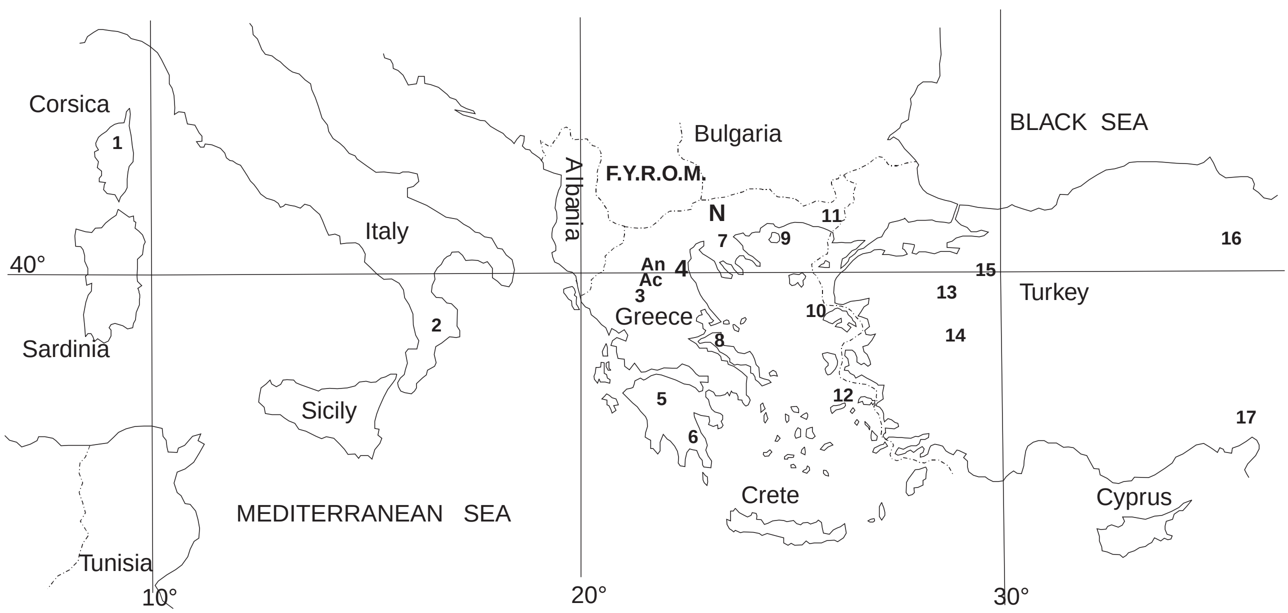

Figure 1. *Pinus nigra* provenances (1...17) and trial sites (N, An, Ac).

from Greece exhibited almost as much variation in growth among themselves as was to be found in all seedlots from the eastern Mediterranean. Nicolic and Tucic [16] also found that heterogeneity within populations is higher than heterogeneity between populations or subspecies; they suggest as possible cause either genetic drift or the heterogeneity of local environments where gene frequencies are controlled through selection.

The reported variability among *Pinus nigra* provenances, especially from the eastern part of its national distribution, has suggested us the establishment of a trial in the oak coppice zone if an extensive reforestation programme were to be undertaken. Such a trial would help to evaluate the performance of the provenances and may possibly correlate the performance differences to original site characteristics, facilitating wider applications.

2. MATERIALS AND METHODS

The trial was established in 1986 on three sites. Two in Anthrakia pilot plantation, Grevena district, Western Macedonia, on conglomerate parent material with clay loam-clay soil, differing only in aspect (south and north respectively), and one in Nigrita pilot plantation, Serres district, Central Macedonia on schist parent material with loam-clay loam soil. The altitude of Anthrakia North is 850 m with NW aspect and slope about 25%; the altitude of Anthrakia South 770 m with S aspect and 15% slope. Nigrita's altitude is about 500 m; the two replications are on the top of a ridge on a flat ground while the third is 30 m lower on a slope of 25% with SE aspect. All sites are in the deciduous oak zone with annual precipitation 600–800 mm, mean monthly temperature from 5 ºC (January) to 25 ºC (August), dry summer and frequent winter or spring frosts. Anthrakia is regarded slightly colder in winter and dryer in summer than Nigrita.

Seventeen provenances in total (*table I*) were tested but, due to the available number of plants, in unequal number per site i.e. Anthrakia South 16, Anthrakia North 12 and Nigrita 13; only 12 provenances were common to all sites. The plants, raised in paper pots in one nursery season (spring–autumn), were planted on prepared soil; the site preparation included sub-soiling, disc-harrowing and tine ploughing.

The trial layout was of randomised block design with the treatments (provenances) arranged in three replications (blocks) per site. In Nigrita the two replications are located in a grassy opening of the oak forest and the third a little further on a weed free spot. The basic unit (plot) consisted of 35 trees planted in 5 rows of 7 trees with spacing 3.5 m between the rows and 1.75 m on rows. The central 15 trees per plot (3 rows and 5 trees per row) were used for recording survival (%), tree height (cm) and diameter at breast height (cm). In total 1845 trees were measured.

Tree survival and height were measured annually. Heavy loss was observed in the two weed infested replications of Nigrita, despite the initial thorough site preparation. Slower tree growth was observed in Anthrakia North. The plot means at age 9 on the sites were used for the subsequent calculations. Survival percentages per plot were transformed with angle (arcsin) transformation prior to statistical analysis, as it is recommended for randomised experiments in which the measurement is a proportion [22].

An analysis of variance per site was performed to identify the best provenances on each site. An overall analysis of the 12 provenances common to the two sites with significant differences was made to investigate site differences and site-provenance interactions. The overall analysis followed the mathematical model:

$$Y_{ikj} = m + S_i + (R_k + r_{ik}) + P_j + (SP)_{ij} + e_{ijk} \text{ where:}$$

Y_{ikj} is the expected value of the j-th provenance, at the i-th site in the k-th replication;

m is the overall mean;

S_i is the effect of the i-th site;

$(R_k + r_{ik})$ is the effect of the k-th replication and the main unit error;

P_j is the effect of the j-th provenance;

$(SP)_{ij}$ is the effect of the i-th site to j-th provenance interaction and

e_{ijk} is the sud-unit error.

In this analysis the sites are tested for significance to the replications and the main unit error, while the provenances and interaction to the sub-unit error when the effects are regarded fixed. In random effects the provenances are tested to the interaction for significance. When the interaction $(SP)_{ij}$ is significant it may further be partitioned with the joint regression technique [2, 9, 11, 17, 21, 24, 31] by regressing the provenances over their site mean into:

$$(SP)_{ij} = b_j S_i + d_{ji} \text{ where:}$$

b_j is the regression coefficient of j-th provenance

d_{ji} the deviation of the j-th provenance in the i-th site and S_i as above.

Table I. Provenances in the trial.

PROVENANCES	Location of seed origin		
Serial number and origin of the seed	Long.	Lat.	Alt.
1. FC06 Noceta, Corsica, France	09 11	42 10	1000
2. IC08 Sila di Katanzaro, Calabria, Italy	16 35	39 16	1200
3. GR31 Malakasi, Kalambaka, Greece	21 17	39 48	1150
4. GR93 Pieria, Katerini, Greece	22 07	40 20	1350
5. GR36 Zarouhla, Aegion, Greece	22 17	37 58	1310
6. GR33 Vamvakou, Parnon, Sparta, Greece	22 34	37 15	1350
7. GR78 Arnea, Chalkidiki, Greece*	23 42	40 34	600
8. GR35 Drymona, Limni, Eubea, Greece	23 68	38 53	600
9. GR80 Thassos isl., Greece	24 34	40 42	550
10. GR79 Lesbos isl., Greece	25 65	39 13	700
11. GR30 Dadia, Soufli, Evros, Greece	25 73	41 80	200
12. GR92 Lazaros, Vourliotes, Samos isl. Greece	26 53	37 48	1035
13. TR21 Golcuk, Alacam, Balikesir, Turkey	28 32	39 26	1370
14. TR18 Saracova, Nazilli, Muggla, Turkey	28 38	38 02	1200
15. TR19 Dagakca, Bursa, Turkey	28 59	40 03	950
16. TR.20 Kosdag, Kagri, Amasya, Turkey	34 22	41 02	1400
17. TR.22 Hizar, Pos, Adana, Turkey	35 15	37 41	1350

*Seedlot from planted stand of unknown origin.

The resulting partitioning of the regressions heterogeneity (b_jS_i) and the remaining (deviations) term in the analysis indicates the contribution of the provenances to the interaction; it is tested for significance to the remainder while the remainder is tested for significance to the residual of the analysis of variance. A regression coefficient b_j close to 1 (the joint regression coefficient), indicates a stable provenance over the environments with minimum contribution to the total interaction.

In the present case with only two sites, this partitioning will lead to invalid regressions consisting of only two points. To overcome this difficulty the partition was realised by regressing the two sites on their mean. The sum of squares (*SS*) in both cases remain the same, but in this case the stability of the *j*-th provenance and contribution to interaction is indicated by the size of its deviations (d_j); the large deviations indicate an unstable provenance with large contribution to the interaction. This contribution can be defined as a percentage of the provenance deviations *SS* to the remainder *SS*, or the total interaction *SS*.

3. RESULTS

The analysis per site has shown highly significant ($p < 0.001$) differences between the provenances in all traits in Anthrakia South and significant differences in diameter ($p < 0.01$) and height ($p < 0.025$) in Nigrita. Significant differences appeared also between the replications, especially in Nigrita, reflecting the variation between the first two weed infested replications and the third one. In Anthrakia North, no differences were found in any trait.

The performance of the provenances on the sites (Anthrakia-South and Nigrita) with significant differences (*tables II* and *III*), show the Corsica (FC06) provenance among the best growers in both sites. Some of the Turkish provenances (TR19, and 21) ranking high at Anthrakia-South are doing less well at Nigrita, while for Thassos (GR80) and partly for Calabria (IC08) provenances the opposite is found. Differences in survival

Table II. Antrakia South: provenance means of height (cm), diameter at breast height (cm) and survival (%).

Provenances	Height	Diameter	Survival
1. FC06 Corsica	302 a	3.9 a	87 cd
2. TR18 Muggla	275 ab	3.8 a	100 a
3. GR79 Lesbos	272 abc	3.7 a	98 ab
4. TR19 Bursa	271 abc	3.8 a	100 a
5. TR.20 Amasya	258 bcd	3.3 ab	91 bcd
6. GR33 Sparta	256 bcd	3.3 ab	98 ab
7. TR21 Balikesir	253 bcd	3.2 abc	96 abc
8. TR.22 Adana	240 bcde	2.7 cd	93 bc
9. GR35 Eubea	233 cd	3.0 bc	100 a
10. GR93 Katerini	233 cd	2.8 bc	98 ab
11. GR36 Aegion	232 cde	2.8 bc	100 a
12. GR30 Soufli	222 def	2.4 cde	96 abc
13. GR78 Arnea	209 ef	2.4 cde	82 d
14. GR92 Samos	190 fg	1.9 de	100 a
15. GR80 Thassos	183 fg	1.6 e	89 cd
16. IC08 Calabria	180 fg	1.5 e	82 d

Note: Means annotated with the same letter do not differ at $p < 0.05$.

Table III. Nigrita: provenance means of height (cm), diameter at breast height (cm).

Provenances	Height	Diameter
1. GR80 Thassos	303 a	4.3 a
2. FC06 Corsica	287 ab	3.4 abc
3. GR36 Aegion	257 abc	3.7 ab
4. GR33 Sparta	248 abc	3.3 abc
5. GR79 Lesbos	245 bc	3.3 abc
6. IC08 Calabria	244 bc	3.1 bc
7. TR.022 Adana	228 cd	2.3 cd
8. TR19 Bursa	224 cd	2.4 cd
9. TR21 Balikesir	219 cd	2.6 bcd
10. GR92 Samos	215 cd	2.5 cd
11. GR35 Eubea	211 cd	2.3 cd
12. GR31 Kalambaka	210 cd	1.9 d
13. GR78 Arnea	186 d	1.8 d

Note: Means annotated with the same letter do not differ at $p < 0.05$.

among provenances appear only on Anthrakia-South, where the lowest survival (82%) was shown by the Calabria (IC08) and Arnea (GR78) provenances.

The overall analysis of the 12 provenances common to Nigrita and Anthrakia-South, (*table IV*) has identified highly significant ($p < 0.001$) differences in height and diameter for replications in sites, provenances and site-provenance interactions. Differences in growth among the sites do not appear significant because of the small number of sites (only 2) and the large mean square (*MS*) of the "Blocks in sites component", against which the sites *MS* were tested (*F*-test) for significance in the analysis of variance. The significant difference between the replications in sites is mainly due to mortality and slow tree growth in the two weed infested replications of Nigrita compared with the third (weed free) replication.

The highly significant ($p < 0.001$) differences in height and diameter identified between the provenances in the over-all analysis, verify the differences found in the per site analysis of variance. The provenance from Corsica (FC06), show the best growth while the Arnea (GR78), Samos (GR92) and Calabria (IC08) provenances are slow growers (*figures 2* and *3*). However, if we assume that these particular sites represent a random sample of the oak zone sites suitable for *Pinus nigra* plantations and consequently the provenance difference is tested against the interaction for significance, the differences between the provenances become insignificant due to the size of the site-provenance interaction.

The highly significant ($p < 0.001$) interaction in height and diameter is mainly due to the different performance of Thassos (GR80) provenance on the sites, as it can be seen in *figures 2* and *3*. It amounts about half of the remaining variation, or 6% of the total variation in the analysis of variance (*table IV*). Thassos (GR80) is doing much better in Nigrita than in Anthrakia-South. To a lesser extent the same applies for Calabria (IC08), while for the Turkish provenances (TR21 and TR19) the opposite is true.

4. DISCUSSION

Differences in early growth among *Pinus nigra* provenances have been almost always observed in trials [1, 12, 13, 18, 32], with different results depending on the sites. Consequently, judging by early growth, different provenances should be selected for different environments, depending on the soil and climatic conditions of the site.

Table IV. Analysis of variance for tree height (*H*) and diameter (*D*) for 12 provenances on 2 sites (Nigrita and Anthrakia South).

Source of Variation	*Df*	*H* (Var. Comp. %)	*D* (Var. Comp. %)	*F*-test denominator
1. Sites (*S*)	1	0	0	2 (*R*)
2. Blocks in Sites (*R*)	3	31***	39***	5 (*Rs*)
3. Provenances (*P*)	11	26***	19***	5 (*Rs*)
4. SxP Interaction	11	24***	23***	5 (*Rs*)
4a Regressions	1	(13)**	(10)*	4b (*Rm*)
4b Remainder (*Rm*)	11	(11)	(13)**	5 (*Rs*)
5. Residual (*Rs*)	45	21	19	
Total	71	100	100	

*** Significant at $p < 0.001$, ** Significant at $p < 0.01$, * Significant at $p < 0.5$.

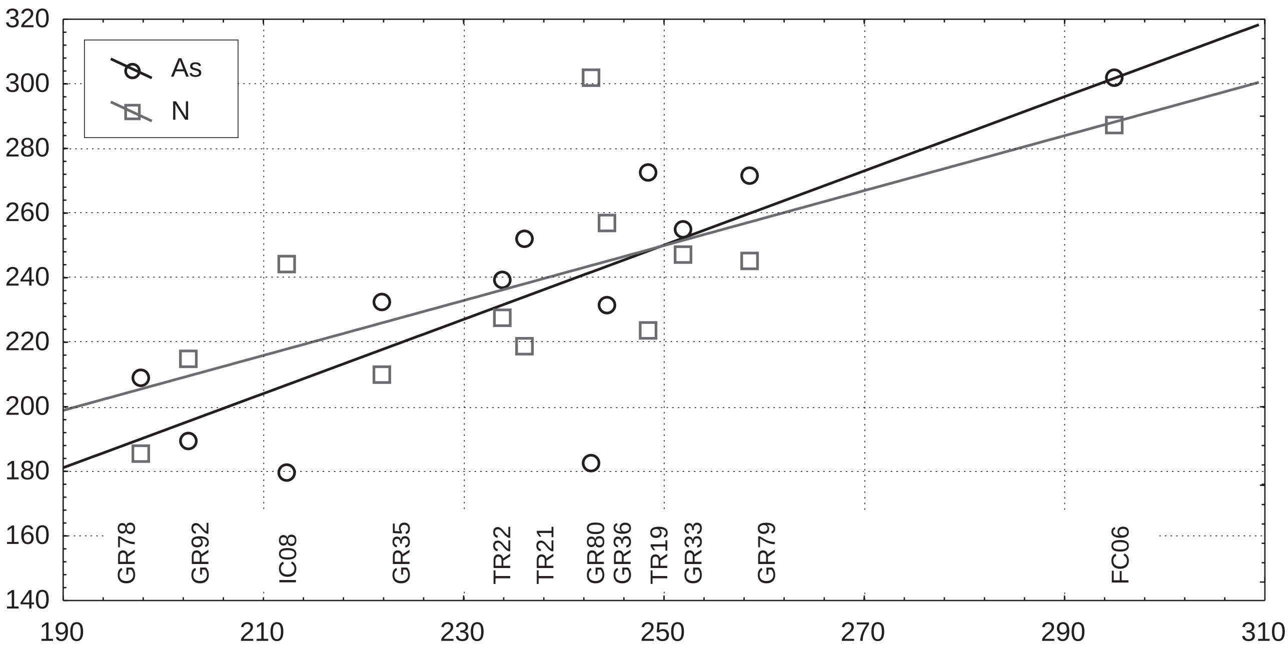

Figure 2. Joint regression of Nigrita (*N*) and Anthrakia South (*As*) heights (cm) on their mean. $As = -37.217 + 1.149x + eps$; $N = 37.271 + 0.851x + eps$.

Provenance differentiation in growth (height, diameter) has only been observed in two out of the three sites of the present trial. In the third site (Anthrakia North) with the slowest growth rates the provenances do not appear to differ significantly. This may mean that provenance growth differentiation is more likely to appear on better sites.

Usually the Corsican provenances have shown high early growth on frost-free sites but low survival, especially on cold sites [8, 19, 23, 25, 26, 30]. The superior growth of the Corsican provenance and its consistent performance over the sites was reported [27] in another trial with 6 provenances (3 western Mediterranean and 3 Greek) on 7 sites in Greece. An earlier evaluation [28] of the present trial has also confirmed the superior growth of the Corsican provenance. The performance of

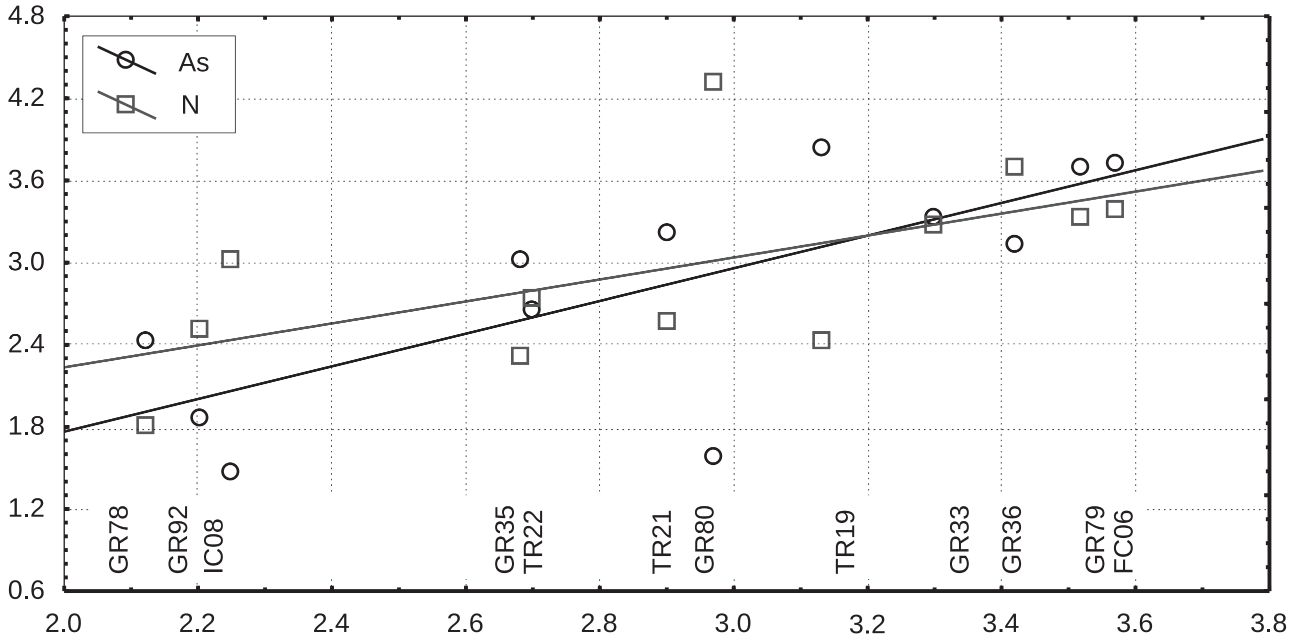

Figure 3. Joint regression of Nigrita (*N*) and Anthrakia South (*As*) diameters (cm) on their mean. $As = -0.623 + 1.194x + eps$; $N = 0.63 + 0.802x + eps$.

the Corsican provenance is consistent with these reports, ranking high in growth but lower (87%) in survival.

Regarding the other provenances, the ranking varies between the sites and this fact is reflected in the strong interaction component in the analysis. Significant site provenance interactions have been frequently reported in *Pinus nigra* provenance trials [29, 30] and were attributed to the broad natural distribution of the species in a wide range of environments, leading to distinct races.

The Thassos GR80 provenance, in fact the only highly interactive provenance, is doing much better in the schist site (Nigrita). It originates from a site of light soil derived from phylosilicate material on metamorphic rocks [7]. The small number of sites (only two in the analysis) does not allow for proper investigation of the possible link between the soil and the observed interaction. Similar performance, although of much lesser extent, is observed in other Greek provenances (GR36, GR92) originating from light soils; for Calabria (IC08), following the same trend, and the Turkish (TR19, TR21) provenances doing better on the heavy soil sites, there are no soil information for their site of origin. Supporting evidence that the interaction in black pine provenances may be linked to the site soil conditions in relation to the site of origin is provided by an older Greek trial [27], where Thassos provenance was doing constantly better on the light soil sites. Thus, it might be possible that performance of the provenances on the two sites be influenced by the site soil factors and the inherited ability of certain provenances to exploit those factors. This leads to accept the suggestion of Nicolic and Tucic [16] that the differentiation of *Pinus nigra* provenances is possibly due to heterogeneity of environments over the areas of its natural distribution, where gene frequencies are controlled by the local environment through selection. It may thus be possible to suggest the existence of soil ecotypes.

5. CONCLUSIONS

The present results show as the best choice in terms of early growth Corsica (FC06) provenance followed by Mugla (TR18), Lesbos (GR79) and Bursa (TR19) for Anthrakia South site. For Nigrita, Thassos (GR80) and Corsica (FC06) are recommended. For Anthrakia North, although Corsica (FC06) ranked first, the choice does not seem to matter. These results are strictly applicable to the specific sites.

For similar sites in the decidous oak zone of northern Greece no recommendations can be safely made, because of the strong site provenance interaction. However the present results and the sited literature support the choice of Corsica (FC05) provenance due to its high and stable performance over the environments. Thassos (GR80) provenance might prove the best choice on light soils, equally good or surpassing Corsica in early growth, but it should be avoided for sites with heavy soils. Both provenances are not among the best in survival, and this should be taken into account for planting in harsh, especially very cold, sites. This factor might partly be overcome by very careful raising of seedlings (larger containers, different substrate) and planting conditions.

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