

Variation in Growth, Flowering and Cone Production in a Clonal Seed Orchard of Aleppo Pine Grown in Greece

By D. MATZIRIS¹⁾

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Summary

In a clonal seed orchard of Aleppo pine, established in 1987, with a total number of 2630 trees, height growth, flowering and cone production were studied and the following results were obtained:

Female flowers appear from the first year after the establishment of the seed orchard but they are low in number and restricted to only few trees of certain clones. The mean numbers of female flowers per tree for all clones were found to be 21 and 56 for the years 1990 and 1991 respectively with ranges from 1 to 59 for the year 1990 and 7 to 136 for 1991. The differences between clones were statistically significant.

Male flowers appeared at age 4 years on a small number of clones (27.6% of the total) and substantially increased the following year (44.7%). Considering the whole seed orchard only 11.2% and 18.3% of trees produced male strobili at ages 4 and 5 years respectively.

Variation among clones in the number of cones produced at ages 9 and 10 years were found to be statistically significant. However, the clone x year interaction effect was insignificant, indicating that high cone producers of one year continue to do so and the following years. The overall mean number of cones/tree of the entire seed orchard was found to be 168 for the year 1995 and 155 for 1996, with ranges among clones from 40 to 348 for the first and 40 to 343 for the second year. Year to year correlation coefficients on clonal averages were very strong for female flower productions ($r = 0.94$) and strong for cone production ($r = 0.74$).

Female flowering and cone production are highly inherited characteristics with broad sense heritability (h^2) estimates of 0.86 and 0.83 for female flowering (1990, 1991) and 0.46 and 0.41 for cone production (1995, 1996).

Key words: *Pinus halepensis*, Aleppo pine, seed orchard, heritability, correlations.

FDC: 165.3; 165.5; 181.521/522; 181.65; 232.311.3; 174.7 *Pinus halepensis*; (495).

Introduction

Aleppo pine (*Pinus halepensis* MILL.) is a species with a circum-Mediterranean distribution (CRITCHFIELD and LITTLE, 1966; MIROV, 1967). The geographic distribution is discontinuous, as it has been strongly affected by past geological and climatic changes and also by human interference. It extends from 9° long. W in Morocco to 36° long. E in Jordan (area of Dibbin) and from 45° lat. N in France to 31° 30' lat. in Israel (SCHILLER *et al.*, 1986). In Asia Minor it is found near Adana and in the Near East it is also found in Syria and Lebanon. The geographic distribution of the species in Greece has been described by PAPAIOANNOU (1935) and is given by PANETSOS (1981).

Aleppo pine hybridises naturally with its related species *Pinus brutia* when they come in contact and natural hybrids have been identified long ago (PAPAIOANNOU, 1954; VIDACOVIC and KRSTINIC, 1974; PANETSOS, 1975, 1981, 1986). Artificial

crosses between the 2 species were performed in 1948 and in 1961 and it has been reported that crossings were successful when *P. brutia* was used as the female parent but not reciprocally (MOULOPOULOS and BASSIOTIS, 1961).

Aleppo pine grows on a variety of forest sites and tolerates different substratums. However it prefers mostly limestone, both marl and chalk. SCHILLER *et al.* (1981) found a distinct relation between the nature of the bed rock and growth of the species. The growth was superior on marl and chalk compared to that on dolomite or limestone. This behavior was attributed to differences in moisture retention capacity by the different substratums. It tolerates high content of free carbonate in the soil and avoids heavy clay soils with poor drainage. It is considered a fast growing species, producing on good sites in Greece (Euboia) up to 10 m³/ha/yr to 12 m³/ha/yr (PANETSOS, 1981). The form of the trees is generally poor due to negative selection practiced for a long time in the past. Aleppo pine forest are found along the coast, at low elevations and were accessible by man from antiquity. In spite of that on Euboia island trees with excellent form and fast growth are still to be found.

Aleppo pine is a resin producing species. There are information (HILLIS, 1987) that resin was used in the Mediterranean region as far back as 3000 years ago, mainly for water proofing and various pharmaceutical purposes. Resin yield is a highly inherited characteristic (MOULALIS, 1991). Ranges among trees from 1 kg to 6 kg per year with an average of 2.7 kg have been reported (GEORGIOULIS, 1964).

The species is very well adapted to the dry mild Mediterranean ecosystem. If flowers at an early age (3rd year) and produces a large number of serotinous cones which persist for many years on the crown of the trees. After fires, the cones open, releasing viable seeds which germinate promptly in the favourable environment, making natural regeneration successful.

A wide network of provenance trials of Aleppo pine, covering nearly the whole geographic distribution of the species have shown that provenance from Euboia island (Greece) was superior in growth in many parts of the world. The superiority was evident in Australia (PALMBERG, 1975), Italy (ECCHER *et al.*, 1982; FUSARO, 1986), Greece (PANETSOS, 1981), Israel (WEINSTEIN, 1989a) and Turkey (TULUKLU *et al.*, 1989). Furthermore this provenance was the best in straightness and more resistant to *Matsococcus joshephi* (MENDEL, 1984). These findings indicate that Aleppo pine is a stable species and that the provenance environment interaction effect is of minimal importance. Therefore the genetic materials found in Euboia island are valuable not only for Greece but also for many other countries within or outside the Mediterranean region.

In 1983 a selection program was initiated in Greece within the best population of Euboia island and in 1987 a 10 ha clonal seed orchard, including 76 clones and a total number of 2630 grafts was established in Amphilochia, west Greece. The purpose of the present study is to evaluate growth, flowering and cone production in the seed orchard and estimate inheritance

¹⁾ National Agricultural Research Foundation, Forest Research Institute, Terma, Alkmanos, 115 28 Athens, Greece

patterns and interrelationships that may exist among the characteristics.

Materials and Methods

The work reported here has been carried out in the clonal seed orchard at Amphiloichia, located in the western part of Greece. This orchard was established in January 1987 and comprises 76 clones derived from intensively selected trees in the natural forests of Euboia. Grafts were 2 year-old at the time of establishment and were planted at a 6 m x 6 m spacing. Clones (1 ramet/clone) were randomly assigned within replications with 1 only restriction. No grafts of the same clone were planted closer than 30 m. The area was an abandoned forest nursery with a deep alluvial fertile soil. Commercial cone harvesting started in the year 1994 when trees were 8 years old.

Height was assessed in the years 1987 to 1991, when trees were 1 to 5 years-old. Female flowers were counted at ages 4 years and 5 years and the number of trees bearing male strobili was recorded in 1990 and 1991 (ages 4 and 5 years). All measurements, except the number of cones, were made in 6 replications. Cones were collected in June from 3 replications and kept and counted separately by tree.

Analyses of variance and calculation of variance components were conducted on the data for each characteristic separately. For cone production a combined analysis was performed for the 2 years examined to establish the year x clone interaction effect. The analyses of female flowers and cone production have been made after square-root transformation of the original data, to normalize the variance (SNEDECOR and COCHRAN, 1967). Variance components and heritability values were estimated following the same procedures applied in an earlier paper (MATZIRIS, 1994).

Results and Discussion

The mean values of the characteristics measured are presented in *table 1*. It is evident from the table that height growth was slow at the beginning (first 2 years) and increased

Table 1. – Total tree height in cm and number of female flowers and cones per tree produced, in a clonal seed orchard of Aleppo pine.

Characteristic	Age Years	Mean	Range	Standard dev.
<u>Height</u>				
1987	1	39	15 - 59	14.107
1988	2	61	33 - 101	14.894
1989	3	172	123 - 245	24.148
1990	4	233	175 - 342	33.942
1991	5	333	258 - 462	42.781
<u>Female flowers</u>				
1990	4	21	1 - 59	13.734
1991	5	56	7 - 136	31.102
<u>Number of cones</u>				
1995	9	168	40 - 348	70.692
1996	10	155	40 - 343	72.422

the following years, when the seedlings were well recovered from the transplanting shock. The mean height of all clones was 39 cm in the year of establishment and increased to 172 cm, 233 cm and 333 cm the following years. The differences between clones were quite high and statistically significant for all years (*Table 2*). At age 5 years (1991) the mean height ranged among the clones from 258 cm to 462 cm, with an overall mean of 333 cm and a standard deviation of 42.7 cm.

It is well known that height growth of grafted seedlings is influenced by the root-stock and that a cloning effect (C effect) is involved. However the values given here are only indicative and show the magnitude of the differences which may be expected in the growth of grafted materials of Aleppo pine. Real differences and the genetic structure of the clones will be reported, in another paper, based on progeny tests that are now 10 year-old and growing under different environmental conditions. It is worth mentioning here that grafting success was high and in contrast to black pine (MATZIRIS, 1982) no problems of graft incompatibility were noticed up to the age of 10 years.

Table 2. – Analyses of variance and broad sense heritability estimates for height (HT), female flowering (FF) and cone number (CN) per tree in a clonal seed orchard of Aleppo pine.

A. SEPARATE ANALYSES

Source	D.F.	Mean Squares $\frac{1}{n}$									
		HT 87	HT 88	HT 89	HT 90	HT 91	FF 90	FF 91	CN 95	CN 96	
Replication	5	1625.336	1902.486	3084.452	3616.894	3628.527	239.525	1744.190	0.185	19.083	
Clones	54	1262.891**	1382.474**	3452.549**	7307.188**	11625.651**	1093.568**	5606.329**	18.338**	17.971**	
Error	270	322.053	437.387	978.384	1724.070	2163.345	150.298	943.853	9.827	10.689	
Broad sense heritability estimates on individual tree basis							0.51	0.45	0.22	0.19	
Broad sense heritability estimates on clone mean basis							0.86	0.83	0.46	0.41	

B. COMBINED ANALYSIS FOR CONE PRODUCTION OVER 2 YEARS

Years	1	0.764
Replications	4	9.624
Clones	54	10.429
Clones x years	54	1.674
Error	216	10.257

¹⁾ **Statistically significant at the 0.01 probability level

Flowering and Cone Production

Flowering

Female flowers appeared from the first year but on few clones only. The second year nearly all the ramets of some clones produced female flowers. At age 4 years all (76) clones had female flowers but only a small proportion of them started to produce male strobili. In this year the variation among clones was very large ranking from 1 to 59 female flowers per tree, with an overall mean for all clones being 21 and the standard deviation being 13.73. In 1991 the production of female flowers was much higher. The range among clones ranked from 7 to 136 flowers per tree with an overall mean of 56 and a standard deviation of 31.1 flowers (Table 1). The analyses of variance (Table 2) showed that differences between clones were statistically significant at 0.01 probability level for both examined years. At ages 4 and 5 years clones bearing male flowers were 27.6% and 44.7% of the total respectively, but the number of strobili were few and not enough for adequate pollinations (Table 3). Only 11.2% and 18.3% of trees were bearing male strobili in the years 1990 and 1991 respectively. The number increased progressively as trees became older and at age 8 years (1994) the commercial maturity of the seed orchard was reached and cone collection started. The results showed that the commercial maturity of Aleppo pine is realized earlier than that of black pine, which requires 10 years from the time of planting (MATZIRIS, 1993). It is well known that the sexual reproduction of Aleppo pine starts at a very early age, with pronounced precocity not only in the initiation of flowering but also in cone yield (WEINSTEIN, 1989b). The precocity of flowering, combined with the serotinous cone habit, resistance to dry conditions and high competitive ability with other woody vegetation, especially at an early age, make Aleppo pine a particularly useful species for the delicate, sensitive to fires, Mediterranean ecosystems. All these advantages favor and enhance its survival under the selection pressure of fires. Being fire dependent it creates by itself a forest floor prone to high flammability so the ecosystems are mainly perpetuated through periodical accidental burning. Under these conditions the species constitutes a more advanced community (climax) as far as stability, complexity and efficiency of energy utilization is concerned (STERN and ROCHE, 1974). Variation among provenances in the time of development of vegetative buds and flowering has been also reported by WEINSTEIN (1989b). It has also been found (WEINSTEIN, 1989c) that trees affected by *Matsococcus josephi* start phenological stages earlier than uninfected trees. As *Pinus brutia* is resistant to this insect, it has been suggested (MENDEL, 1984; LIPHSCHITZ and MENDEL, 1987) that it is to be preferred for reforestation projects in environment, sensitive to *Matsococcus josephi*.

Cone Production

Variation among clones in their annual cone production is evident from the analysis of variance (Table 2). The differences are very large and statistically significant for both examined

years. The mean number of cones per tree varied from 40 (clone 78) to 348 (clone 74) for the year 1995 (age 9 years), with overall mean 168 cones. At age 10 years the range was from 40 (clone 78) to 343 (clone 47) with an overall mean of 155 and standard deviation 72.4 cones. The mean number of seed per cone was found to be 25 at age 7 years, while at age 10 years it increased to 100. The corresponding percentages of sound seeds were 67.4% and 87% respectively. These differences are attributed to variation in the amount of pollen production and to environmental conditions prevailing at the time of pollen dispersal.

The variation among clones in cone production indicates that they are not contributing equally to the seed crop, as is required in an idealized seed orchard. At age 9 years, 25% most abundant cone producers, produced 39.2% of the total cones, while 25% least productive clones produced only 12.3% of the total cones. Similar was the trend at age 10 years (1996), with 25% best producers yielding 41.6% of cones. This disproportionate contribution of Aleppo pine clones to the cone crop is in agreement with results reported for Scots (BHUMIBHANON, 1978; JONSON et al., 1976), black (MATZIRIS, 1993) and loblolly (North Carolina State University, 1976) pine seed orchards as well as for Norway spruce (SWEET, 1975) and Douglas fir (EL-KASSABY et al., 1984). A combined analysis of variance for cone production over the 2 years (Table 2), showed that the F value for the clone x year interaction effect was not significant. Although the ranking of clones changes from year to year, the best cone producers are at the top for both years and the less productive clones in the bottom. These results are in agreement with those found in other coniferous species, such as Scots pine (JONSON et al., 1976) *Pinus virginiana* (SCHMIDTLING, 1983) and black pine (MATZIRIS, 1993).

The degree of departure from the equal cone production of all clones for the two years examined is shown in figure 1. The closer the curves approaches to the ideal situation presented by the straight (diagonal) line, the higher is the probability that each clone will contribute to the same extent to the maternal gene pool in the seed orchard.

Interrelationship Between Characteristics

PEARSON and SPEARMAN's rank correlation coefficients between all combinations of the characteristics studied are shown in table 4. As expected year to year correlations for height growth were strong between consecutive years and their values gradually decrease, as the time between measurements increases from $r = 0.89$ to $r = 0.40$. The values of SPEARMAN's rank correlations were similar. The relationships between the number of female flowers produced in 1990 and the tree height at different ages were in all cases weak, with r values ranking from 0.17 to 0.31 while the corresponding values for the year 1991 were ranked from 0.16 to 0.25. Since one of the main objectives of breeding and selection of trees is to increase vegetative growth together with flowering, the relationship between these characteristics is of interest. Correlations were also low and insignificant between cone production and height

Table 3. – Number of clones and trees with male strobili (in 6 single tree replications of 76 clones) in the years 1990 (4 years) and 1991 (5 years), clonal seed orchard of Aleppo pine in Amphilochia, Greece.

Year	Age	Clones with No.	male flower. %	Trees with No.	male flower %
1990	4	21	27.6	50	11.2
1991	5	34	44.7	82	18.3

and cone production in Aleppo pine are under strong genetic control.

Conclusions

From analyses of height growth, flowering and cone production in a clonal seed orchard of Aleppo pine grown in Greece the following conclusions can be drawn:

1. There are significant differences among clones in height growth. However the clone x year interaction effect for cone production is insignificant, indicating that good cone producers in one year continue to be good in the following years.

2. At age 4 and 5 years all ramets of clones produce female flowers, while the percentage of clones bearing male strobili was only 27.6% at age 4 years and 44.7% at age 5 years. This delay influences the commercial maturity of the seed orchard.

Year to year correlation in the number of female flowers ($r = 0.94$) and in the number of cones ($r = 0.74$) are very strong.

3. Female flowering and cone production are strongly inherited characteristics which need attention in the establishment and management of Aleppo pine seed orchards.

4. 25% of the best cone producers produce 39.2% of the total crop cones, while the 25% of the least productive clones only 12.3%. This disproportional contribution must be taken into consideration in the management of the seed orchards.

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