

MEDITERRANEAN STONE PINE PROVENANCES' PERFORMANCE IN INNER SPAIN - LOW DIFFERENTIATION IN HEIGHT GROWTH AND SHOOT PHENOLOGY.

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Abstract - In 1994, a stone pine provenance trial of the FAO Committee *Silva Mediterranea* started by exchange of seed lots covering the natural range of the species. In Spain, 34 *Pinus pinea* provenances from Spain (17), France (5), Lebanon (4), Turkey (4), Portugal (2), Greece (1) and Italy (1) were planted at four experimental sites. The objective of the trial was to study geographic or ecologic differentiation between provenances in adaptative traits such as survival, vegetative and reproductive phase change, phenology and growth. No differences in survival or phase change were observed between provenances in the first decade: not even the warmest coastal provenances suffered any frost damage (at -17° C). The observed height and diameter growth patterns of all provenances at each site was quite similar (Variation coefficient VC 6-10% between provenances at each site), though the proverbial phenotypic plasticity of the species produced strong spatial autocorrelations of the response variables, both between and within sites (spatial VC 16-26% at each site). This impeded any standard data analysis based on the hence failed experimental designs, a nearest-neighbours adjustments resolving the problem. Comparison of the adjusted average heights of each provenance at the four sites by principal component analysis showed a common trend between sites (first component, variance proportion 0.48) of a more vigorous growth in plants from colder inland provenances, coinciding with an earlier spring shoot phenology and a stronger tendency to polycyclism.

Key words: Mediterranean stone pine, provenance trial, growth phenology.

INTRODUCTION

Stone pine is one of the most characteristic trees of the Mediterranean flora, adapted to dry sandy or rocky soils. Scattered populations of the species range from Portugal to Turkey and Lebanon along the Mediterranean coasts, though the most extensive native forests grow in the Iberian Peninsula, where humans (or hominids) have gathered the cones of the Mediterranean Stone pine for their large edible kernels for at least 50 000 years (Badal, 2001). Few tree species are better adapted than Stone pine to the severe summer drought, harsh winters and poor eroded soils (especially sand dunes, granite or active limestone) that characterise Mediterranean Spain.

A life-history singularity of Stone pine is its reproductive strategy that lacks the aerial seed bank in serotinous cones typical of the other sympatric Mediterranean pines (Tapias *et al.*, 2004), but is based on dyszoochory of the large, wingless seeds (0.5-1 g) by rodents or birds. The costs of predator swamping result in a biomass allocation to cones similar or even superior to bole growth in adult Stone pine stands (Cabanettes and Rapp, 1981).

In consequence of the clumped secondary dispersion to distanced seed caches, its population structure and dynamics are not based on massive post-fire regeneration events like in other sympatric pines that present dominant single-cohort stands, but on scattered dispersal of small seed lots by vertebrates throughout the more or less open Mediterranean woodlands (Rodrigo *et al.*, 2004). In its most genuine habitats, excessively drained, deep sand layers or dunes as well as bare rocky slopes, understorey is scarce

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and therefore crown fires are rare in the isolated growing pines. Stone pine is also present in many holm-oak or cork-oak dominated forests, though only scattered as single, overlooking trees or groups of few trees, similarly to *Pinus sabiniana* Douglas in the Californian blue oak woodlands. Incidentally, Stone pine seedlings are more shade-tolerant than those of Maritime or Aleppo pines (Awada *et al.*, 2003) and can delay the vegetative phase change from juvenile to adult growth habit and needle type during several years, in order to survive in limiting environments - an adaptative trait similar to Canaries pine (Klaus, 1989; Climent *et al.*, 2006).

Few studies have focussed on the phenotypic or molecular differentiations between populations or geographic areas in the range of Stone pine, differences that have been found to be minor than in other pine species (Fallour *et al.*, 1997; Court-Picon *et al.*, 2004; Gordo *et al.*, 2007; Vendramin *et al.*, 2008). In this context, the objective of the present paper was to estimate the relevance of geographic or ecologic differentiation between seed sources in adaptative traits evaluated in common garden experiments: survival, vegetative and reproductive phase change, growth and phenology.

MATERIAL AND METHODS

The analysed provenance trial started in 1994 with an exchange of seed lots between Mediterranean countries within the natural Stone pine range, on an initiative of the French INRA within the framework of the FAO Committee *Silva Mediterranea* (Martín and Prada, 1995). In Spain, four experimental sites were planted between end of 1995 (at Tordesillas, Cucalón and Quintos de Mora) and beginning of 1997 (Trespaderne) with one and two-year old nursery raised seedlings respectively (Table 1) from 34 seed sources in

Spain (17), France (5), Lebanon (4), Turkey (4), Portugal (2), Greece (1) and Italy (1).

The trials were measured several times since establishment, though height growth will be analysed here only at the age of nine years in three sites. Due to severe deer-browsing damages, the fourth site (Quintos de Mora) was measured only two years later, when the saplings had recovered growth after fencing. Stem diameter at ground level was highly correlated with individual tree height (r 0.95), thus results will centre only on height. The observed height and diameter growth patterns of all provenances at each site were not only quite similar (Variation coefficient VC 6-10% between provenances at each site), but moreover the proverbial phenotypic plasticity of the species produced strong positive spatial autocorrelations of the response variables, both between and within sites (spatial VC 16-26% at each site) that impeded any standard data analysis based on the hence failed experimental designs. A nearest-neighbours adjustment by the iterated Papadakis method (Bartlett, 1978) was applied in order to estimate a local mean as proxy of microsite effects on tree height (Mutke *et al.*, 2007; Mutke *et al.*, 2010).

For phenology monitoring, in 2001 220 saplings from eight ecologically contrasting provenances in Lebanon, Turkey, France, Spain and Portugal were marked in two sites (Tordesillas and Cucalón). During spring flush (March-June 2001), apical shoot was measured twice a month, recording also the current reproductive phenostages if male or female cones were present at the tree. Individual shoot elongation was adjusted by non-linear regression at thermal time scale (degree-day sum from 1st March) (Mutke *et al.*, 2003), in order to analyse the variance of the regression coefficients of the adjusted Gompertz model among provenances.

	Tordesillas	Cucalón	Trespaderne	Quintos Mora
Province	Valladolid	Castellón	Burgos	Toledo
Altitude	670 m	660 m	560 m	827 m
Latitude	41° 29' N	39° 47' N	42° 48' N	39° 24' N
Longitude	4° 57' W	0° 38' W	3° 23' W	4° 04' W
Annual rainfall (mm)	472	592	742	400
Mean temperature (°C)	12.2°	13.9°	10.8°	13.4°
Experimental Design	Incomplete Blocks	Incomplete Blocks	Complete Blocks	Incomplete Blocks
Provenances	34	34	29	29
Blocks	51	68	15	43 + 1
Plantation date	February 1996	December 1995	February 1997	April 1996
Grid	2.5 x 4 m	2.5 x 4 m	2 x 3 m	2.5 x 4 m
Surviving plants	1 759 from 1 836	2 161 from 2 448	1 110 from 1 305	953 from 1 566

Table 1. Spanish test sites of the stone pine provenance trial.

RESULTS

State of the trial

The performance of all provenances was quite similar at each site, though with the before-mentioned strong microsite effects on tree growth. The vegetative phase change (transition from juvenile growth to adult growth habit, with fixed cyclic growth and secondary needles) took place in the third or fourth year in the two faster-growing of the four sites, only some year later in the other ones. Also reproductive phase change was achieved in 23% (female) and 27% (male) of the saplings with 6-9 years in the first two plots, but in none plant in the latter two due to their delayed development. Both vegetative and sexual maturity were closely linked to plant size, and no significant size-independent seed source effects were observed. Survival was about 85-96% in three sites, only 61% at the deer-browsed Quintos de Mora, though without significant differences between provenances. Especially no frost damage was observed in any plants (not even from the southern coastal provenances), though e.g. in Tordesillas, minimum temperatures reached an absolute minimum of -17°C and late frosts of -4°C in May (during plain spring growth flush).

Height growth

The 34 accessions differed significantly in height growth, although the differences were masked by the strong environmental heterogeneity, as well between sites as within each of them (Table 2). The spatial adjustment at each site incremented the degree of genetic determination (differences between provenances at each site), in height growth from 2-12% before spatial adjustment to 5-40% once adjusted (attributing 65-80% of the overall variation to the spatial covariable). Anyway, a weighted joint regression, which took into account Site and Provenance as factors and the estimated local mean at each tree position as covariable at each site, attributed 64% of the total sum of squares to differences between sites (factor that includes the fact that one site was 2 years older when measured), another 23% to within-site microenvironment effects and only 1% to differences between provenance means (though highly significant, F-value 19.8). The resting variation among plants within provenances, except that attributable to microsite effects, was the residual of the adjusted model, about 11%. Until the age of nine years, most

provenances from the Spanish inland, one from northern Portugal and another French one performed best, whereas coastal provenances from Southern Turkey, Southern Spain and the French Côte d'Azur grew less. The ranking of other provenances from Portugal, Greece or Northern Turkey was not stable between sites, and some seed sources, such as the only Italian one, were not present at all sites.

Phenology monitoring

The results of the phenology analysis grouped the provenances again differing a Northern group with colder Inland seed sources (Portugal, Inner Spain, Lebanon) from the southern coasts (Southern Spain, Turkey). The latter group needed a slightly higher degree-day sum for shoot development (timing parameter b -the moment of maximum spring growth- at 920 degree days above 1°C after 1st March versus 870 dd in the Northern group) with no significant differences between provenances of each group. Anyway, there was a high variability among the trees of each provenances related to plant vigour estimated by the stem cross-section area and an important residual variance among individuals of each provenance not explained by the provenance or tree size (R^2 was only 0.21; Table 3). Slope parameter c did not differ significantly between groups or provenances.

CONCLUSIONS

Though a significant trend of a more vigorous growth, an earlier spring shoot phenology and a stronger tendency to polycyclism in plants from some colder inland provenances was observed, these geographic or ecotypic differentiations result to be very scarce compared to phenotypic plasticity (common response of all provenances to environmental variation between or even within test sites). This result runs in parallel to the low degree of genetic differentiation found through molecular markers (Vendramin *et al.*, 2008).

LITERATURE

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Country	Provenance	H9 TOR (cm)	H9 CUC (cm)	H9 TRE (cm)	H11 QUI (cm)	Phenology
GR	Chalkidiki Agios Nicolaos	251	173	67	127	
I	Feniglia 67	238	228	-	-	
RL	Beit Mounzer (Mad. J.)	241	198	-	-	x
RL	Kornaale	223	190	55	118	
RL	Qsaibe	227	192	-	-	
RL	Bkassine	212	182	-	-	
TR	Natalia-Kumluca-Kalmaz	199	174	61	118	x
TR	Yalova-Dumanlidag	249	185	52	119	
TR	Canakkale-Eceabat	235	190	60	124	
TR	Mugla-Yatagan-Katraci	219	159	64	102	
F	St. Raphaël (Var)	198	175	66	128	
F	Villeneuve (Hérault)	221	174	68	116	
F	Saintes-Maries (B.du Rh.)	230	192	72	111	
F	St. Aygulf (Var)	220	186	72	126	
F	Brignoles- Le Val (Var)	242	204	70	120	
P	Alcacer do Sal	226	183	66	110	
P	Serra do Minho	246	186	69	115	x
E	Cogeces de Iscar	248	197	74	124	
E	Valorio1	245	202	78	118	
E	Valorio2	258	216	75	124	
E	Toro	235	211	85	126	
E	Vega Sicilia	238	211	71	120	
E	Íscar	237	200	70	132	x
E	Tordesillas	235	192	72	115	x
E	Portillo	219	179	67	119	
E	Cadalso	239	205	76	113	
E	Tarazona de la Mancha	245	193	71	118	
E	Doñana	207	173	65	117	
E	Las Lomas	223	190	61	129	
E	Cartaya	207	192	62	117	x
E	Parafrugell	232	195	74	111	
E	Biar	226	193	66	130	x
E	Garrovillas	221	170	66	122	
E	Budia	231	172	-	-	
Site mean		230	190	68	120	

Table 2. Adjusted mean tree height at 9 years of 34 tested provenances at Tordesillas, Cucalón and Trespaderne test sites, at 11 years at Quintos de Mora. The spring phenology of eight marked provenances was studied.

Source of variance	Sum of Squares Type I	d.f.	Mean Square
Group	23,208	1	23,208 ***
Prov.(Group)	4,214	5	843 NS
Block	5,737	3	1,912 NS
Stem cross-section area	21,794	1	21,794 ***
Group x c.-s. area	6,405	1	6,405 NS
Residuals	231,894	89	2,606
Total	293,253	100	

Table 3. Analysis of variance for location parameter b of the individual shoot growth model

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