

# TO GROW OR TO SEED: ECOTYPIC VARIATION IN REPRODUCTIVE ALLOCATION AND CONE PRODUCTION BY YOUNG FEMALE ALEPPO PINE (*PINUS HALEPENSIS*, PINACEAE)<sup>1</sup>

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Age and size at the first reproduction and the reproductive allocation of plants are linked to different life history strategies. Aleppo pine only reproduces through seed, and, as such, early female reproduction confers high fitness in its infertile highly fire-prone habitats along the Mediterranean coast because life expectancy is short. We investigated the extent of ecotypic differentiation in female reproductive allocation and examined the relation between early female reproduction and vegetative growth. In a common-garden experiment, the threshold age and size at first female reproduction and female reproductive allocation at age seven differed significantly among Aleppo pine provenances of ecologically distinct origin. Significant correlations among reproductive features of the provenances and the ecological traits of origin were found using different analytical tools. In nonlinear models of cone counts vs. stem volume, medium-sized trees (not the largest trees) produced the highest cone yield, confirming that, at the individual level, early female reproduction is incompatible with fast vegetative growth. The contribution of founder effects and adaptation to contrasting fire regimes may be confounding factors. But considering all traits analyzed, the geographical patterns of resource allocation by Aleppo pine suggest ecotypic specialization for either resource-poor (favoring early reproduction) or resource-rich (favoring vegetative growth) habitats.

**Key words:** age at first reproduction; cones; Mediterranean; Pinaceae; *Pinus halepensis*; reproductive allocation; reproductive effort; threshold size.

The trade-offs between resource allocation to reproduction vs. vegetative structures (reproductive allocation) and the relationship between vegetative growth and current reproduction (reproductive effort) are “hot” topics in botanical research, justified by its relevance to plant adaptation (reviewed by Bazzaz and Ackerly, 1992; Obeso, 2002). Following the principles of plant allometry (the relative growth of a part in relation to an entire organism), the differential allocation of resources to reproduction, storage, defense and growth throughout ontogeny is highly specific for species survival strategy (Wilson, 1983; Niklas and Enquist, 2002, 2003). In contrast with the abundant literature on reproductive cost in angiosperms (from herbaceous to woody species), conifers have been relatively neglected (with a few exceptions; see Despland and Houle, 1997; Silvertown and Dodd, 1999). Moreover, the majority of studies on conifers tend to focus on wild populations, while the only data published on intraspecific genetic variation in reproductive traits comes from a few seed-orchard studies (Matziris, 1997, 1998).

Aleppo pine (*Pinus halepensis* Mill.) is one of the most thoroughly studied Mediterranean conifers, especially with respect to vegetation dynamics under drought and fire distur-

bances (Daskalakou and Thanos, 1996; Ne’eman and Trabaud, 2000; Ne’eman et al., 2004). As an obligate seeder living in fire-prone habitats, early reproduction is crucial for the species’ fitness, not only in its native habitat, but also as an allochthonous invader in some regions (Barbero et al., 1998; Richardson and Higgins, 1998). Extremely precocious cone bearing by Aleppo pine compared to other pines enables it to accumulate a huge aerial seed bank in serotinous cones at a young age. This seed bank ensures its resilience against stand-replacing cycles occurring at intervals of 30 yr or less (Agee, 1998; Thanos and Daskalakou, 2000; Tapias et al., 2001), but very frequent fires can favor its replacement by seeder shrubs (Pausas and Lloret, 2007). Thinnings in postfire stands have been seen to reduce the time and height for first reproduction (De Las Heras et al., 2007).

Like many other pine species, young Aleppo pines start their reproductive life as females, producing little or no pollen until their crown is structurally well developed. Additionally, in a young stand ovulate strobili contribute much more to reproductive success than do male strobili because efficient pollination is usually accomplished by nearby mature stands (Ne’eman et al., 2004). Hence, we can readily accept that early female reproduction has a higher adaptive value than early male reproduction in Aleppo pine. Considering that high early reproductive effort has a cost for later growth and reproductive success (Obeso, 2002), we suggest that different regional combinations of resource availability and disturbance regime across Aleppo pine’s distribution, have exerted contrasting intraspecific selection pressures on early female reproduction. Both age and size thresholds for female reproduction and the size for maximum reproductive output are relevant when studying the environmental and genetic effects on reproductive precocity in this species because size and not cellular senescence have been reported

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to account for a great part of ageing phenomena in different tree species (Mencuccini et al., 2005).

High genetic differentiation among populations has been reported in Aleppo pine for neutral (Agúndez et al., 1999; Gómez et al., 2001, 2005) and adaptive traits (Atzmon et al., 2004; Chambel et al., 2007; Voltas et al., in press), suggesting that both nonselective genetic processes and directional selection have contributed significantly to its geographical genetic structure.

We hypothesize that (1) directional selection has caused ecotypic differentiation for female reproductive allocation in Aleppo pine, and (2) at the individual level, assuming that early female reproduction occurs at the expenses of vegetative growth, we would not expect that good growers are the best seeders. To test these hypotheses, we used data collected over nine years in a common-garden experiment containing 53 provenances covering most of Aleppo pine's range of distribution.

## MATERIALS AND METHODS

**Plant material**—The common-garden experiment included 53 autochthonous provenances from continental Spain, the Balearic Islands, France, Italy, Greece and Tunisia, that we further classified into 15 ecologically distinct regions based on both their environmental similarity and geographical proximity (Gil et al., 1996) (Fig. 1, Appendix 1). The geographical range was from 23°54' E to 4°50' W, and from 36°30' N to 43°24' N, with an altitudinal range from sea level (in some of the Greek, Italian, and Balearic provenances) up to 1238 m a.s.l. (in southern Spain). Open-pollinated seeds from 20 to 30 trees spaced at least 100 m apart were obtained from each population and subsequently bulked into population seedlots. One-year-old seedlings grown in containers were produced following standard nursery practices in 1997.

Climatic variables for each provenance were obtained from the Global Climatic Model WorldClim implemented in the program DIVA-GIS version 1.3 (Hijmans et al., 2005) at a 30" resolution. We chose six variables with recognized influence on ecotypic variation of Mediterranean pines (Tapias et al., 2004; Voltas et al., in press): annual mean temperature (T), annual precipitation (P), annual summer precipitation (Ps, precipitation of the warmest quarter, corresponding to June, July and August), and temperature annual range (TAR, equivalent to the maximum temperature of warmest month minus the minimum temperature of coldest month) to characterize each provenance and ecological region. Other variables putatively relevant were found to be closely correlated to these six variables.

**Study site**—The trial site was a with a 5% slope located at 39°49'29"N and 00°34'22"W at 640 m a.s.l., within the species natural range in Spain. Soil type was a shallow calcixerollic xerochrept of about 0.5 m depth. Seedlings were planted in a randomized complete block design, with four blocks per provenance and four plants per block (16 trees per provenance) planted at 2.5 × 2.5 m spacing. Mean annual rainfall at the site is 472 mm (22% falling in summer) and mean annual temperature is 13.8°C. The site was chosen as a good representative of Aleppo pine's ecological niche in Spain, where both climate and soil conditions are moderately limiting. Furthermore, neighboring mature stands acted as complementary pollen sources, ensuring pollination and thus preventing cone abortion (Goubitz et al., 2002). Mortality was negligible (less than 5%) and occurred mostly during the first 3 years of growth.

**Variable description**—Height was recorded at ages four, seven, and eight years, and basal diameter was recorded at ages four and seven years. Cones were counted yearly from age three (1999) to age nine (2005), covering the transition from mostly vegetative to mostly reproductive (female) individuals. Concomitantly, the occurrence of any multiple female flowering events within the same morphogenetic cycle (Pardos et al., 2003) was recorded as a binary variable (Mf), either the presence or absence of this character in the crown. Individual age (Aff) and height (Hff) at first female flowering were obtained from annual cone counts and periodic height measurements.

Reproductive allocation of each tree, as the static measurement of the ratio of reproductive biomass to total standing biomass (Obeso, 2002), was studied at age seven (summer of 2003), coinciding with a sharp increase in the number of reproductive individuals. We chose stem volume over bark (Vob) as a surrogate for total standing biomass, in preference to height or basal area (Reinhardt et al., 2006). Vob was calculated by the following equation, assuming the stem to be conical:

$$Vob = \frac{\pi}{12} Db^2 H, \quad (1)$$

where  $Db$  is basal diameter and  $H$  is total tree height.

The biennial cycle of cone development in Aleppo pine (as in most other pines) implies that every year, two strobili cohorts, from two consecutive flowering events, are growing together in the crown. Even when cones usually remain in the crown for more years (serotinous or xeriscent), their dry mass does not increase after their second winter. Considering that these two cohorts compete for the same resources, we defined the variable cone count at age seven (Cc) as the sum of all immature ("green") cones at that age (Fig. 2A, B). We therefore considered reproductive allocation (RA) as the ratio  $Cc/Vob$ , in number of cones per cubic decimeter. We also checked for any differences in mean cone dry mass between provenances and for a possible trade-off between cone count and individual cone biomass, by collecting and oven-drying one cone per

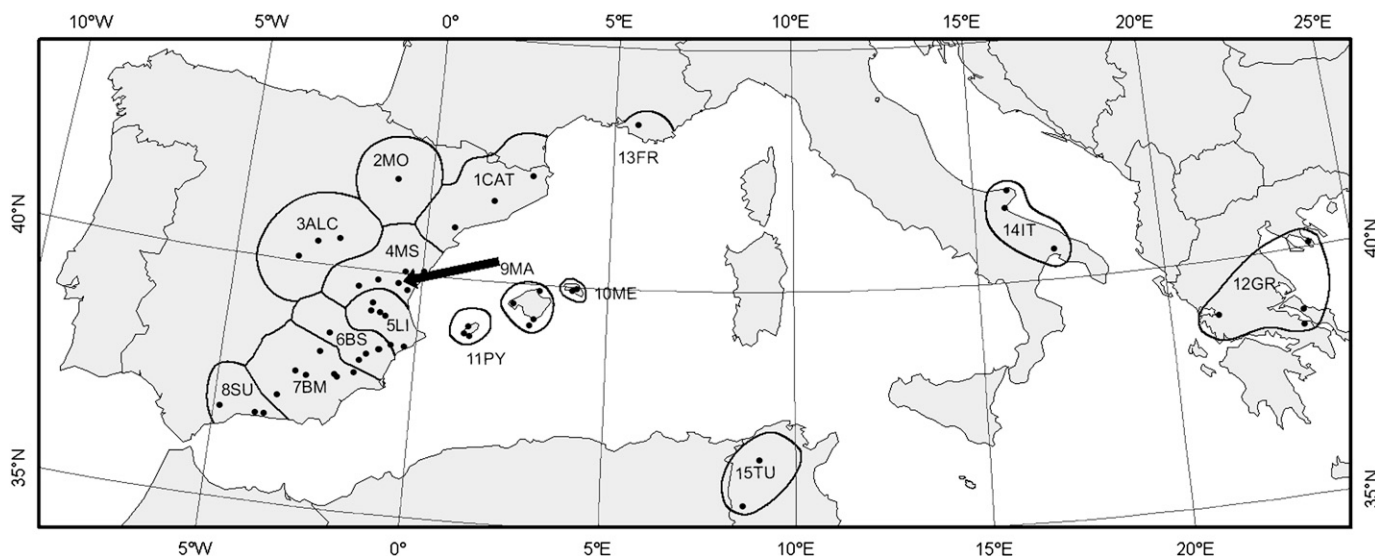


Fig. 1. Location of the 53 studied provenances of *Pinus halepensis* (black dots), grouped into 15 ecological regions (each represented by a perimeter and acronym). Arrow points to the trial site.



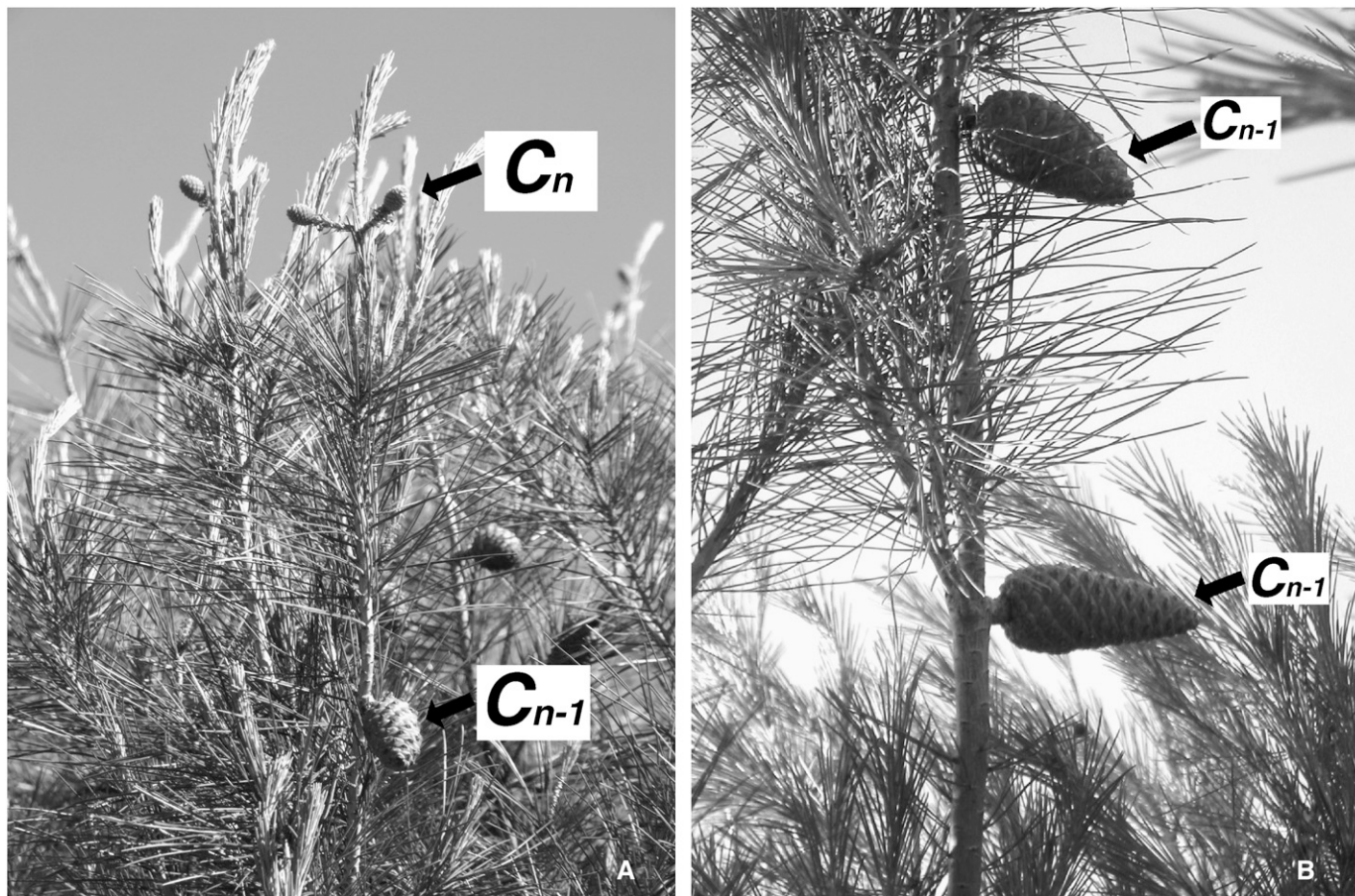


Fig. 2. (A) Cone cohorts integrating the variable  $C_c$ , in early summer for *Pinus halepensis*: current year's female strobili ( $c_n$ ), and previous year's crop ( $c_{n-1}$ ). (B) Multiple flowering events from the same calendar year are a common trait in this species; therefore, all green cones, either  $c_n$  or  $c_{n-1}$  were considered.

tree from 352 trees (44% of the total, belonging to all 53 provenances). Because differences between provenances or regions were insignificant and the relationship of cone dry mass to cone counts at the individual level was barely significant and positive (hence, abundant cone production in a tree was associated with bigger, rather than smaller cones), we accepted  $C_c$  as a realistic surrogate of female reproductive biomass.

**Data analysis**—We analyzed variation in the target variables  $V_{ob}$ ,  $A_{ff}$ ,  $H_{ff}$ ,  $M_f$ ,  $C_c$ , and  $RA$  at the different scales, ecological region and provenance within ecological region, using a general linear model with the following structure (all effects considered fixed):

$$Y_{ijk} = \mu + R_i + P_j R_i + B_k + \varepsilon_{ijk}, \quad (2)$$

where  $Y$  is the dependent variable,  $\mu$  is the general mean,  $R$  is the additive effect of ecological region,  $P$  is the additive effect of the provenance within ecological region,  $B$  is the additive effect of the block, and  $\varepsilon$  is the error term. We also considered an alternative model with  $V_{ob}$  as a covariate to adjust for the size-dependent variation of  $RA$  (Klinkhamer et al., 1992), but this effect was nonsignificant; hence, we decided to stick with the simpler model. Groups of equal means were discriminated using Tukey's unequal  $N$  honestly significant difference (HSD) test (significant at the  $P < 0.05$  level). We used the ratios of sums of squares to compare the proportion of variation explained by each factor.

**Nonlinear modeling of reproductive allocation**—To complement the analysis of  $RA$  as a quotient, we assessed the relationship between the production of female cones and tree size and how this relationship differed geographically by modeling  $C_c$  as a function of  $V_{ob}$ . Although the literature suggests that there is

a consistently positive correlation between vegetative and reproductive biomass irrespective of size (Klinkhamer et al., 1992; Despland and Houle, 1997; Niklas and Enquist, 2003), we checked two alternative relations. (1) Asymptotic. There is an inflection point and a theoretical maximum for reproductive output. Larger trees have more cones, but above a given tree size, there is no further increase in cone production. This relation is expressed by the family of sigmoidal ascending curves. (2) Ascending-descending, with a maximum value. The larger cone crops do not correspond to the larger trees. This relation is expressed by the family of functions with a maximum and an inflection point.

Several biparametric functions of each family were evaluated among those given by (Ratkowsky, 1990). In addition to adjusting the model for pooled individual data, we assumed that observations from trees sharing a common geographical origin were correlated (ecological regions or provenances). This relation was examined in linear and nonlinear mixed models (Lindstrom and Bates, 1990; Davidian and Giltinan, 1995), in which fixed effects were common to the whole meta-population and random effects were specific for each ecological region. Practically, in the first step, both effects ( $a$  and  $b$ ) were assumed to be mixed, divided into a fixed part and a random part, specific to each geographical unit (henceforth referred to as  $u$  and  $v$ ). If convergence was not attained in this way, only one of the effects was considered mixed, while the other was assumed to be fixed. Model comparisons were based on two times the log-likelihood and the sum of squared errors. Smaller estimates for both statistics indicated a more accurate model. Models were fitted by restricted maximum likelihood methods using the MIXED (linear models) or SAS macro NLINMIX (nonlinear models) procedures in the program SAS version no. 8.2 (SAS Institute, Cary, North Carolina, USA). We assessed systematic deviation from the meta-population level model due to each geographical unit using EBLUP (empirical best linear unbiased predictor) for the random effects.

The family of ascending–descending curves gave the best-fit models to explain the observed reproductive allocation patterns. Giving preference to the two times the log-likelihood statistic, we selected the model represented by the equation:

$$Cc = a(0.1 \cdot Vob)^{b-0.1 \cdot Vob} \quad (3)$$

To favor convergence, Vob was transformed to  $0.1 \cdot Vob$ . This model implies that the largest cone counts were not produced by the biggest trees, but at  $Vob_{max} = -1/b$ . The maximum cone count, corresponding to this volume, will be referred to as  $Cc_{max}$ . Differences between models were tested using pairwise comparisons in which the complete structure (an independent model for each geographical origin) was compared with the reduced structure (the model is fitted to the pooled data from the two origins) in terms of minus two times the log likelihood.

**Relation with geographical and climatic variation**—To assess the relation between phenotypic traits and geographical and climate parameters, we used adjusted means for each provenance after removal of block effects and parameters derived from nonlinear models at the scale of ecological regions. Two methodological approaches were applied to characterize the climate of each provenance in the common garden experiment. First, we used the scores of the first two principal components of the climate variables from a principal component analysis. And second, we used the pooled environmental distance between each provenance and the trial site (therefore, calibrating climate data, following Rutter and Fenster [2007]). Gower's distance (GD) was used for this purpose in the formula:

$$GD = \frac{1}{p} \sum_{i=1}^p \frac{|A_i - B_i|}{r_i} \quad (4)$$

where  $p$  is the number of environmental characteristics,  $A_i$  and  $B_i$  are the values of those environmental characteristics at the two compared sites and  $r_i$  is the total range of the environmental characteristic in the dataset.

## RESULTS

**Variability among individuals for stem growth and reproductive features**—Considering all populations together, mean age at the first flowering event (Aff) was 6.1 yr and ranged from 3 to 9 yr. The threshold height for female flowering (Hff) ranged from 45 to 444 cm with a mean of 163 cm. Reproductive allocation (RA) ranged from 0 to 7.8 cones/dm<sup>3</sup> with a mean of 0.5 cones/dm<sup>3</sup>.

**Geographical variation for vegetative and reproductive traits**—All the studied variables differed significantly among ecological regions and among provenances within ecological regions ( $P < 0.005$ ), except for Mf and Cc, which showed marginally nonsignificant provenance effects (Table 1). The influence of ecological region was especially high for Cc, RA, and Hff, while provenances within ecological regions contributed greatly to the variation of stem volume (Vob). Block effects were significant ( $P < 0.05$ ) for all variables except for Cc and Mf, but contributed little to the variation in Vob and RA. Moreover, the proportion of variation explained by geographical origin (ecological region plus provenance) was highest for RA at 39.2%, contrasting with only 18.9% for stem volume.

The Tunisian 15TU and the Iberian 2MO and 7BM regions stood out because of their high RA and their young age and small size at first flowering (Aff and Hff; Table 2). These two regions diverged from two of the Balearic ones (11PY and 9MA), Catalanian (1CAT), and Greek (12GR) for female reproductive traits. Surprisingly, the other Balearic ecological region, Menorca (10ME), started female reproduction at a relatively small size and had a moderately high RA. For stem volume (Vob), only the fast-growing Greek provenances differed significantly from the others (Table 2).

TABLE 1. ANOVA table (GLM) for the six phenotypic variables analyzed in *Pinus halepensis*: Vob, stem volume over bark; Aff, age at first flowering; Hff, height at first flowering; Mf, presence of multiple flowering cycles; Cc, count of all immature cones at the crown; RA, reproductive allocation (Cc/Vob). Factors considered as fixed are R, ecological region; P (R), provenance within ecological region; B, block; and  $\varepsilon$ , error. Last column is the percentage of the variation explained by each factor, based on sum of squares.

Variable	Factor	df	SS	MS	F	P	Var. expl. (%)
Vob	R	14	9794.2	699.6	4.796	0.0000	7.8
	P(R)	37	13919.0	376.2	2.579	0.0000	11.1
	B	3	1409.4	469.8	3.221	0.0223	1.1
	$\varepsilon$	700	102097.8	145.9			81.2
	Total	754	127625.8				
Aff	R	14	87.2	6.2	4.750	0.0000	7.9
	P(R)	37	71.0	1.9	1.460	0.0388	6.4
	B	3	32.7	10.9	8.320	0.0000	3.0
	$\varepsilon$	700	917.2	1.3			83.1
	Total	754	1107.5				
Hff	R	14	576573.0	41184.0	12.609	0.0000	17.5
	P(R)	37	279360.0	7550.0	2.312	0.0000	8.5
	B	3	143252.0	47751.0	14.620	0.0000	4.3
	$\varepsilon$	700	2286316.0	3266.0			69.4
	Total	754	3298371.0				
Mf	R	14	4.259	0.304	4.343	0.0000	7.7
	P(R)	37	3.479	0.094	1.342	0.0870	6.3
	B	3	0.169	0.056	0.803	0.4924	0.3
	$\varepsilon$	700	49.038	0.070			88.8
	Total	754	56.909				
Cc	R	14	33508.2	2393.4	13.986	0.0000	20.8
	P(R)	37	7627.7	206.2	1.205	0.1914	4.7
	B	3	545.9	182.0	1.063	0.3640	0.3
	$\varepsilon$	700	119794.9	171.1			74.5
	Total	754	161508.7				
RA	R	14	97.35	6.95	17.069	0.0000	23.7
	P(R)	37	22.73	0.61	1.508	0.0287	5.5
	B	3	4.31	1.44	3.530	0.0146	1.1
	$\varepsilon$	700	285.16	0.41			69.5
	Total	754	410.56				

**Nonlinear modeling of reproductive allocation**—The selected model is represented by the equation

$$Cc = (48.0630 + u) (0.1 \cdot Vob)^{[-1.1479 + v] (0.1 \cdot Vob)}, \quad (5)$$

where  $u$  and  $v$  are random effects dependent on the ecological region. Considering all ecological regions together,  $Vob_{max}$  was 8.7 dm<sup>3</sup>, corresponding to percentile 67.8. There were large differences among the specific models for each ecological region (Table 3, Fig. 3). While for some provenances (2MO, 8SU, 15TU) the maximum cone yields occurred at low stem volumes, others approximated an asymptotic relationship (14IT, with  $Cc_{max}$  corresponding to volumes close to the maximum values). The values of the quotient  $Cc_{max}/Vob_{max}$  (Table 3) quantify the steepness of the ascending part of the curve (Fig. 3); hence, it gives a good graphical idea of the maximum reproductive allocation ( $RA_{max}$ ). This parameter,  $RA_{max}$  was tightly correlated to RA ( $r = 0.94$ ,  $P < 0.001$ ); hence, the ranking for ecological regions was highly conserved between both parameters. Regions 15TU (Tunisia) and 2MO (inland Ebro Depression), together with 8SU (southern Spain) had similar high values for  $RA_{max}$ , and contrasted sharply with the other ecological regions (Table 3). At the other extreme, the Balearic 11PY (Ibiza) and 9MA



TABLE 2. Multiple range tests (unequal *N* HSD) at the ecological region scale for mean values of the six phenotypic variables analyzed: Vob, stem volume over bark; Aff, age at first flowering; Hff, height at first flowering; Mf, presence of multiple flowering cycles; Cc, count of all immature cones at the crown; RA, reproductive allocation ( $Cc / Vob$ ). Means with the same letter are not significantly different at  $P < 0.05$ .

Region	Vob	Region	Aff	Region	Hff	Region	Mf	Region	Cc	Region	RA
2 MO	16.8 a	15 TU	5.25 a	15 TU	101.6 a	1 CAT	0.000 ab	3 ALC	4.1 a	11 PY	0.17 a
3 ALC	19.0 a	10 ME	5.53 ab	2 MO	117.2 ab	11 PY	0.000 ab	1 CAT	5.3 ab	1 CAT	0.26 ab
15 TU	19.7 a	8 SU	5.90 abc	7 BM	140.0 ab	4 MS	0.033 ab	15 TU	5.8 ab	9 MA	0.28 ab
5 LI	20.6 a	2 MO	5.93 abcd	10 ME	145.0 abc	9 MA	0.036 ab	10 ME	7.3 ab	12 GR	0.34 ab
4 BS	21.3 a	5 LI	5.93 abc	5 LI	147.7 ab	6 BS	0.053 ab	9 MA	9.2 abc	13 FR	0.40 abc
7 BM	22.0 a	7 BM	5.93 abc	3 ALC	152.0 b	10 ME	0.067 ab	4 MS	9.3 abc	4 MS	0.42 abc
9 MA	23.3 a	13 FR	5.93 abcd	8 SU	156.5 bc	13 FR	0.067 abc	12 GR	9.9 abc	6 BS	0.43 abc
1 CAT	23.7 a	6 BS	6.07 abcd	6 BS	157.6 bc	8 SU	0.071 ab	5 LI	10.4 abc	3 ALC	0.51 abc
6 BS	23.9 a	12 GR	6.12 abcd	13 FR	163.7 abcde	7 BM	0.088 ab	11 PY	10.4 abc	10 ME	0.58 abc
13 FR	24.1 ab	14 IT	6.16 abcd	4 MS	165.6 bcd	3 ALC	0.091 ab	13 FR	13.1 abc	5 LI	0.61 abc
8 SU	24.3 ab	1 CAT	6.28 bcd	1 CAT	178.5 bcd	5 LI	0.092 ab	14 IT	14.5 bc	14 IT	0.65 bc
10 ME	25.7 ab	4 MS	6.28 bcd	14 IT	179.2 bcd	12 GR	0.140 abc	2 MO	14.8 abc	7 BM	0.65 bc
14 IT	26.1 ab	3 ALC	6.30 bcd	9 MA	193.8 cde	2 MO	0.143 abc	8 SU	17.4 abc	8 SU	0.88 c
11 PY	26.9 ab	9 MA	6.62 cd	12 GR	203.1 de	14 IT	0.200 bc	6 BS	17.9 c	2 MO	0.95 abc
12 GR	33.1 b	11 PY	6.89 d	11 PY	226.8 e	15 TU	0.344 c	7 BM	37.9 d	15 TU	2.07 d

(Majorca), 1CAT (Catalonia) and Greek (12GR) were tightly grouped with the lowest  $RA_{max}$ . The pairwise model comparisons confirmed the significance of this grouping.

**Geographical and ecological correlations**—The two principal components had eigenvalues greater than one and accounted for 78.06% of the variation of climate variables. The first principal component (PC 1) had an especially high and positive load for TAR and negative loads for P and Ps (Table 4). This component had significant negative correlations (Table 5) with traits linked to flowering precocity (Aff and Hff), and a subsequent positive correlation with traits linked to abundant cone yield at nine years (Mf, Cc, RA and  $RA_{max}$ ). Gower's distance, indicating climatic relatedness between each seed origin and the trial site, correlated with reproductive features in a similar way as described for PC 1, but with lower coefficients. Euclidean geographical distance and longitude (represented by X utm, the X value of Universal Transverse Mercator [UTM] coordinates) were the only variables more correlated to vegetative growth (Vob) and volume for maximum reproductive output ( $Vob_{max}$ ) than to reproductive features. This

correlation indicated an increase of growth toward eastern Mediterranean that does not correspond to the climate variation analyzed. Worthy of note, RA had a higher correlation with Gower's distance than with geographical distance, indicating an ecotypic variation for female reproductive allocation (Table 5).

## DISCUSSION

Our study is one of few to demonstrate intraspecific variability for reproductive allocation in a tree species (Aleppo pine). The use of a common-garden experiment (a provenance trial covering most of the species natural area) allowed us to avoid the environmental bias inherent to field studies. Given that our data are based on many provenances tested at a single-site, instead of a reciprocal transplant experiment, we used the calibration to the site conditions as suggested by Rutter and Fenster (2007) to facilitate the comparison of local and increasingly more distant provenances or ecological regions, both geographically and ecologically.

**Relation between reproduction and vegetative growth**—Non-linear models of cone production vs. stem volume revealed that, among young, same-aged Aleppo pines, larger cone crops do not correspond to larger trees. Medium-size trees produced the highest cone yields (an average 67.8 percentile for stem volume). This trend is not apparent in the published literature (Klinkhamer et al., 1992; Despland and Houle, 1997; Niklas and Enquist, 2003) and was consistent among ecological regions and provenances (data not shown at provenance level). This supports that precocious and intense female reproduction is incompatible with a high vegetative growth in Aleppo pine. However, there was a significant positive autocorrelation among successive cone counts in our study, suggesting that at the individual level, intense current reproduction does not lead to a significant loss of future reproduction (fecundity cost). This lack of fecundity cost confirms previous observations that periodic intense mast years do not occur in Aleppo pine (Tapias et al., 2001) and contrasts with the behavior of many other tree species, including the closely related Mediterranean stone pine, *Pinus pinea* (Kelly and Sork, 2002; Mutke et al., 2005b), but see (Knops et al., 2007). Analyses at different within-tree modular levels (branch, shoot, and meristems)

TABLE 3. Empirical best linear unbiased predictor (EBLUP) values per ecological region for the random parameters in Eq. 4 ( $u$ ,  $v$ ), coordinates of the maximum cone count value ( $Cc_{max}$  and  $Vob_{max}$ , volume over bark corresponding to  $Cc_{max}$ ) and values of the maximum reproductive allocation,  $RA_{max}$  ( $Cc_{max}/Vob_{max}$ ).

Region	$u$	$v$	$Cc_{max}$	$Vob_{max}$ (dm <sup>3</sup> )	$RA_{max}$
1CAT	-27.3637	-0.0398	6.41	8.420	0.80
2MO	35.8771	0.0194	27.36	8.861	3.05
3ALC	-5.4991	-0.0799	12.75	8.145	1.60
4MS	-15.7869	-0.0549	9.87	8.314	1.21
5LI	0.8041	-0.2123	13.22	7.352	1.78
6BS	-10.2839	-0.0833	11.29	8.122	1.41
7BM	-6.7639	0.2892	17.69	11.646	1.47
8SU	40.9026	-0.5554	19.21	5.871	3.19
9MA	-24.8862	-0.0635	7.04	8.255	0.88
10ME	-5.4991	0.1963	16.45	10.509	1.59
11PY	-30.4457	-0.0547	5.39	8.315	0.67
12GR	-17.9355	0.1690	11.32	10.216	1.13
13FR	-9.4676	0.0261	12.66	8.914	1.45
14IT	4.4845	0.5056	30.10	15.569	1.77
15TU	71.8632	-0.0617	36.47	8.267	4.25

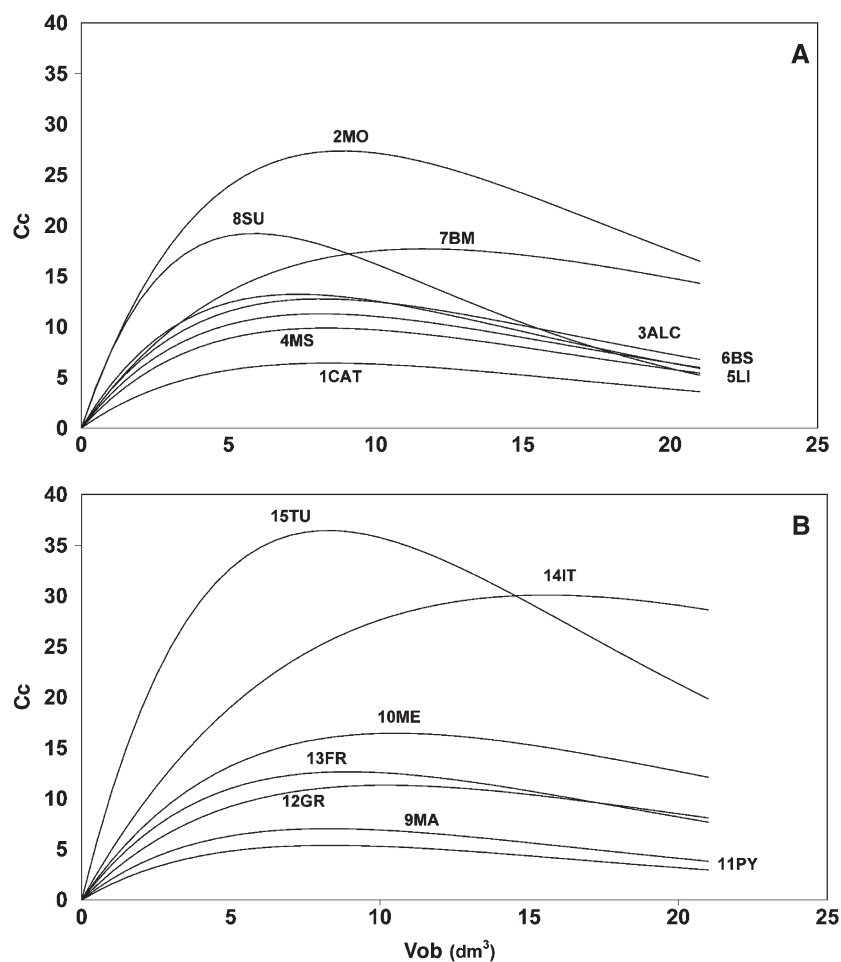


Fig. 3. Models of reproductive allocation for each ecological region of *Pinus halepensis* based on Eq. 5. (A) (B) Vob, volume over bark; Cc count of all immature cones at the crown (see Fig. 2). Codes for the ecological regions are in Appendix 1.

should help to unveil the developmental and physiological processes underlying reproductive costs and putative compensation mechanisms.

**Ecotypic differentiation for growth and female reproduction in Aleppo pine**—Both GLM analysis and specific nonlinear models obtained through EBLUP confirmed our first hypothesis that female reproductive allocation differs significantly among Aleppo pine ecological regions. Because the age and size thresholds for cone bearing (Aff and Hff) were strongly (negatively) correlated with high female reproductive allocation

TABLE 4. Principal component loadings for the four climate variables analyzed: mean annual temperature (T), temperature annual range (TAR), annual precipitation (P) and annual summer precipitation (Ps, precipitation of the warmest quarter, corresponding to June, July and August).

Variable	PC 1	PC 2
T	−0.329	−0.909
TAR	0.872	0.325
P	−0.641	0.324
Ps	−0.744	0.503

tion at age seven, the patterns of variation for these three variables were highly coincident. While the minimum age at maturity or age of first reproduction (Aff) has been found to have a high evolutionary significance in woody plants (Keeley and Zedler, 1998; Verdú, 2002), the height for first reproduction seems more related to the environmental variation (expressed by the first principal component of climate variables, Table 5). This higher differentiation for the size threshold for reproduction is coherent with the recent postulation that size is more closely related to the ontogenetic shifts in functional processes than cellular senescence in many tree species (Mencuccini et al., 2005).

Worthy of note, both the first principal component of climate variables and, to a lesser extent, the ecological distance between each provenance and the trial site were significantly correlated with traits linked to precocious and/or abundant reproductive output. In particular, RA was more related to Gower's distance than to geographical distance, confirming an ecotypic variation for female reproductive allocation.

Plants from the Tunisian ecological region and some Iberian regions (from Ebro basin and southernmost Mediterranean coast) had a marked female reproductive precocity and high reproductive allocation compared with plants from Ibiza,

TABLE 5. Correlations between phenotypic, geographical and environmental variables at (A) the provenance level ( $N = 53$ ) and, for the parameters derived from nonlinear models, at (B) the (ecological region level ( $N = 15$ )). Correlation coefficients and significance level are in italics. Correlations with  $P < 0.05$  are shown in boldface. Vob, stem volume over bark; Aff, age at first flowering; Hff, height at first flowering; Mf, presence of multiple flowering cycles; Cc, count of all immature cones at the crown; RA, reproductive allocation (Cc/Vob). PC 1 and 2, principal components of climate variables; GD, Gower's ecological distance between the seed source and the trial site; Dist\_km, Euclidean geographical distance between the seed source and the trial site; X utm (Universal Transverse Mercator), Y utm, Alt (altitude), geographical coordinates at the center of the mean point of each seed source.

Variable	PC 1	PC 2	GD	Dist_km	X utm	Y utm	Alt
A) Provenance means							
Vob	-0.2123 <i>0.0635</i>	-0.1366 <i>0.1648</i>	0.070 <i>0.309</i>	<b>0.436</b> <i>0.001</i>	<b>0.461</b> <i>0.000</i>	0.210 <i>0.066</i>	<b>-0.371</b> <i>0.003</i>
Aff	<b>-0.2932</b> <i>0.0166</i>	0.0127 <i>0.4640</i>	-0.174 <i>0.107</i>	-0.122 <i>0.193</i>	-0.046 <i>0.371</i>	<b>0.241</b> <i>0.041</i>	-0.146 <i>0.149</i>
Hff	<b>-0.4300</b> <i>0.0007</i>	-0.1513 <i>0.1398</i>	-0.080 <i>0.284</i>	0.217 <i>0.059</i>	<b>0.314</b> <i>0.011</i>	<b>0.374</b> <i>0.003</i>	<b>-0.448</b> <i>0.000</i>
Mf	<b>0.2407</b> <i>0.0413</i>	0.0900 <i>0.2607</i>	<b>0.234</b> <i>0.046</i>	<b>0.340</b> <i>0.006</i>	<b>0.299</b> <i>0.015</i>	-0.098 <i>0.242</i>	0.040 <i>0.387</i>
Cc	<b>0.3082</b> <i>0.0124</i>	-0.0654 <i>0.3208</i>	<b>0.261</b> <i>0.029</i>	<b>0.255</b> <i>0.033</i>	0.186 <i>0.091</i>	<b>-0.246</b> <i>0.038</i>	0.031 <i>0.414</i>
RA	<b>0.3339</b> <i>0.0073</i>	-0.0307 <i>0.4135</i>	<b>0.263</b> <i>0.029</i>	0.109 <i>0.219</i>	0.025 <i>0.431</i>	<b>-0.356</b> <i>0.004</i>	0.143 <i>0.153</i>
B) Ecological region means							
Vob <sub>max</sub>	-0.1117 <i>0.3459</i>	-0.0010 <i>0.4986</i>	-0.212 <i>0.224</i>	<b>0.523</b> <i>0.023</i>	<b>0.561</b> <i>0.015</i>	<b>0.460</b> <i>0.042</i>	0.226 <i>0.209</i>
Cc <sub>max</sub>	0.4211 <i>0.0590</i>	0.0043 <i>0.4939</i>	0.117 <i>0.339</i>	0.340 <i>0.107</i>	0.247 <i>0.187</i>	-0.122 <i>0.333</i>	-0.282 <i>0.155</i>
RA <sub>max</sub>	<b>0.5160</b> <i>0.0245</i>	0.0415 <i>0.4416</i>	0.293 <i>0.145</i>	0.108 <i>0.351</i>	-0.035 <i>0.451</i>	-0.396 <i>0.072</i>	-0.371 <i>0.087</i>

Majorca, Catalonia, and Greece. Across its range of distribution, Aleppo pine has an increasing reproductive allocation and diminishing threshold age and size from east to west and a less marked increasing reproductive allocation toward the south. This geographical pattern mimics an increase in temperature and annual temperature range and a reduction in annual and summer rainfall toward the western and southern edges of the distributional range. An adaptive shift from vegetative to reproductive meristems in response to stressful or resource-poor environments for southern and western provenances (extreme temperatures, lower rainfall, and shallow soils) can be invoked to explain this trend, similarly to that reported for *Arabidopsis* (Bonser and Aarssen, 2001, but see Sakai et al., 2006).

The high ecotypic differentiation for reproductive features among provenances contrasts with a low differentiation for vegetative growth, represented by stem volume. Only the Greek provenances stood out from the rest, surprisingly similar to each other. This outlier behavior of fast-growing Greek provenances has been corroborated in different common garden experiments (Matziris, 2000; Chambel et al., 2007). The lack of significant ecotypic variation for growth in Aleppo pine parallels findings in other Mediterranean pines, such as the Canary Island pine (*Pinus canariensis*) (López et al., 2007) and the Mediterranean stone pine (*Pinus pinea*) (Mutke et al., 2007, 2008) and suggests a low fitness value of vegetative growth per se in these pines living in disturbed and seasonally dry environments. This intriguing common pattern should be studied considering the trade-offs between resilience (either through resprouting or sexual reproduction) and growth (Pausas et al., 2004; Schilck and Ackerly, 2005).

**Alternative interpretations and perspectives**—There are two alternative causal factors for the geographical variation in early reproduction by female Aleppo pines. One is an effect of fire

regime, based on the known adaptive value of early and intense reproduction against stand-replacing fires in obligate seeders (Schilck and Ackerly, 2001). Lightning fires are rare in the eastern Mediterranean, but in the western part of Aleppo pine's distribution they are quite frequent (Vázquez and Moreno, 1998; Ne'eman et al., 2004). The lower levels of serotiny in natural Aleppo pine provenances from Greece and Israel compared to those from the Iberian Peninsula have been postulated to correspond to a higher adaptation to fire in the western part of the species range (Tapias et al., 2001). Hence, the westward decrease in the threshold size for reproduction shown by our experiment could also reflect divergent evolution related to different natural fire regimes, as proposed for other taxa (Zammit, 1987; Keeley and Zedler, 1998; Climent et al., 2004; Tapias et al., 2004).

Our second alternative explanation for geographical variation in female reproduction is population founder effects. Considering that high reproductive allocation is typical of colonizing or expanding populations (Obeso, 2002), the divergence of reproductive features in Aleppo pine can be interpreted with respect to the species postglacial dynamics in the Mediterranean basin. The relatively recent (8000–10000 yr) spread of *Pinus halepensis* following a general migration from northeast to southwest has been well established using various biochemical and DNA markers (Barbero et al., 1998; Agúndez et al., 1999; Gómez et al., 2005). The coincidence of colonization routes and climatic gradients hampers separating between founder effect and local ecotypic adaptation. The island populations along the Mediterranean can perhaps provide a good sampling system to further confirm the extent of founder effects in the geographical variation of Aleppo pine.

We are aware that this work opens many research fronts. Further research is needed to understand the evolutionary interplay between contrasting fire regimes and early reproductive allocation in this fire-resilient species, and male reproduction should

also be considered at higher developmental stages. This need is urgent, considering the increased frequency of catastrophic forest fires during the last few decades across the range of Aleppo pine and the predictions of more severe droughts and higher temperatures for the Mediterranean basin. Outside the species' natural range, this knowledge will help to establish better controls to minimize its invasive potential.

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APPENDIX 1. Region (Reg.) and main climate characteristics of the studied provenances (Prov.) of *Pinus halepensis*. Alt: Altitude; T: mean annual temperature; TAR: annual temperature range; P: annual rainfall; Ps: summer rainfall (June, July and August).

Reg. code	Region	Prov. code	Provenance name	Longitude	Latitude (N)	Alt (m a.s.l.)	T (°C)	TAR (°C)	P (mm)	Ps (mm)
1 CAT	Catalonia	11	Cabanellas	2°47'00"E	42°14'51"	221	14.6	23.6	720	158
1 CAT	Catalonia	21	Tivissa	0°50'06"E	42°19'46"	368	14.8	25.3	587	122
1 CAT	Catalonia	31	Sant Salvador de Guardiola	1°45'48"E	41°40'22"	399	14.4	23.1	628	163
2 MO	Ebro Depression	61	Zuera	0°55'18"W	41°55'05"	576	12.0	27.4	474	101
3 ALC	Southern Plateau	82	Valdeconcha	2°52'19"W	40°26'55"	779	12.8	29.9	433	62
3 ALC	Southern Plateau	83	Alcantud	2°18'49"W	40°33'48"	1004	10.8	30.4	505	95
3 ALC	Southern Plateau	84	Colmenar de Oreja	3°20'00"W	40°05'25"	716	13.7	31.0	433	49
4 MS	Iberian Range	91	Cirat	0°28'08"W	40°03'01"	473	14.6	24.6	440	94
4 MS	Iberian Range	92	Tuéjar	1°09'33"W	39°49'06"	665	13.2	27.2	424	83
4 MS	Iberian Range	93	Enguadanos	1°38'54"W	39°38'26"	950	11.9	29.8	485	91
4 MS	Iberian Range	102	Cucalón	0°36'50"W	39°47'26"	608	13.8	25.2	472	97
4 MS	Iberian Range	111	Benicasim	0°01'33"E	40°04'37"	449	14.6	23.8	523	109
4 MS	Iberian Range	112	Gilet	0°20'55"W	39°26'03"	171	16.6	22.8	451	88
5 LI	East Spain	101	Tibi	0°38'55"W	38°31'08"	993	13.1	27.4	546	89
5 LI	East Spain	103	Villa de Ves	1°14'57"W	39°10'39"	857	13.1	28.5	462	80
5 LI	East Spain	104	Jarafuel	1°00'40"W	39°09'43"	592	14.8	27.0	428	74
5 LI	East Spain	105	Bicorp	0°51'30"W	39°06'12"	619	14.7	26.6	454	80
5 LI	East Spain	109	Commercial seed	—	—	429	15.2	27.4	386	66
5 LI	East Spain	131	Villajoyosa	0°18'14"W	38°29'44"	98	17.7	25.4	422	65
6 BS	N. Betic Mts.	141	Ricote	1°25'52"W	38°08'31"	734	14.8	29.1	398	54
6 BS	N. Betic Mts.	142	Monovar	0°57'27"W	38°23'05"	760	14.0	28.1	467	72
6 BS	N. Betic Mts.	143	Monovar	0°55'35"W	38°23'34"	701	15.1	27.9	416	64
6 BS	N. Betic Mts.	144	Paterna	2°16'37"W	38°37'40"	1014	12.8	31.3	470	60
6 BS	N. Betic Mts.	145	Abaran	1°15'49"W	38°16'32"	629	14.9	28.8	403	59
7 BM	S. Betic Mts.	151	Quentar	3°24'47"W	37°13'40"	1238	12.8	31.7	578	39
7 BM	S. Betic Mts.	152	Benamaurel	2°44'19"W	37°42'05"	914	14.1	30.4	452	44
7 BM	S. Betic Mts.	153	Velez Blanco	2°00'55"W	37°47'26"	799	14.1	29.2	416	49
7 BM	S. Betic Mts.	154	Santiago de la Espada	2°28'03"W	38°13'35"	761	14.1	30.9	432	48
7 BM	S. Betic Mts.	156	Lorca	1°32'04"W	37°51'51"	766	14.1	28.8	427	57
7 BM	S. Betic Mts.	157	Alhama de Murcia	3°01'19"W	37°45'10"	818	15.1	30.6	449	39
7 BM	S. Betic Mts.	158	Quesada	1°56'50"W	37°44'52"	706	15.1	28.5	373	43
8 SU	South Spain	171	Lentegi	3°41'15"W	36°49'20"	507	16.7	26.1	378	16
8 SU	South Spain	172	Carratraca	4°50'01"W	36°50'32"	643	15.4	26.3	695	29
8 SU	South Spain	173	Frigiliana	3°55'13"W	36°49'03"	583	15.7	27.2	456	20
9 MA	Majorca	182	Palma de Mallorca	2°56'26"E	39°08'56"	46	16.7	21.9	563	77
9 MA	Majorca	183	Ses Salines	3°02'49"E	39°17'05"	15	16.8	21.8	568	79
9 MA	Majorca	184	Alcudia	3°10'12"E	39°52'20"	143	15.7	21.7	704	103
9 MA	Majorca	185	Son Martí/Calviá	2°29'13"E	39°35'14"	272	15.8	22.3	526	91
10 ME	Menorca	186	Alcotx	4°10'05"E	39°58'16"	93	16.6	21.1	613	90
10 ME	Menorca	187	Atalix	4°03'12"E	39°54'53"	33	16.9	21.1	608	89
11 PY	Ibiza	191	Cala d'hort	1°14'45"E	38°53'14"	215	15.9	22.6	542	85
11 PY	Ibiza	192	Ses Salines	1°23'54"E	38°50'26"	10	17.5	22.1	443	70
11 PY	Ibiza	193	Ses Salandres	1°19'55"E	39°02'50"	133	17.1	22.1	467	78
12 GR	Greece	211	Istaia-eyboia	23°30'46"E	38°44'29"	27	17.5	27.2	506	35
12 GR	Greece	212	Amfilohia	21°17'38"E	38°52'54"	260	14.2	29.0	975	69
12 GR	Greece	213	Tatoi-attica	23°28'12"E	38°26'49"	152	16.3	27.6	552	38
12 GR	Greece	214	Kassandra	23°54'27"E	40°05'13"	173	14.4	27.7	510	67
13FR	France	221	Gemenos	5°40'00"E	43°24'60"	196	12.4	25.0	707	101
14 IT	Italy	231	Litorale Tarantino	17°07'04"E	40°37'08"	106	15.2	25.5	551	76
14 IT	Italy	232	Gargano Monte Pucci	15°56'34"E	41°54'06"	188	14.2	22.7	524	87
14 IT	Italy	233	Gargano Marzini	3°31'??"E	41°32'47"	0	16.1	26.3	472	76
15 TU	Tunisia	241	Thala	8°39'00"E	35°33'60"	527	14.9	33.6	467	63
15 TU	Tunisia	242	Tabarka	9°04'31"E	36°30'18"	144	17.8	31.5	558	51