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Influence of Stand and tree attributes and silviculture on cone and seed productions in forests of *Pinus pinea* L. in northern Tunisia

Boutheina Adili, , Philippe Balandier

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Mediterranean Stone Pine for Agroforestry

Edited by:
S. Mutke, M. Piqué, R. Calama



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Mediterranean Stone Pine for Agroforestry



Editors: S. Mutke, M. Piqué, R. Calama

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The FAO-CIHEAM Interregional Cooperative Research Network on Nuts



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The FAO-CIHEAM Interregional Cooperative Research Network on Nuts (FAO-CIHEAM Nut Network) was established in 1990, after an expert consultation organized by FAO (REU, RNE and AGPS). Exchange of scientific information, joint applied research, exchange of germplasm and establishment of links between researchers were identified as the main objectives. In 1996, FAO and CIHEAM agreed to co-sponsor the Network. CIHEAM was already involved in fostering research activities on nut trees.

The Network structure is based on a Coordination Centre (Coordinator and Secretary), supported by different Sub-networks (Working Groups), each one with its liaison officer and the mission of fostering and coordinating specific activities. IRTA Mas de Bover (Spain) has been the Coordination Centre from the start of the Network activities in 1990. Today, the Network has 7 Sub-networks (6 tree-crop species and 1 miscellaneous): Almond, Chestnut, Hazelnut, Pistachio, Stone Pine, Walnut, all of them having a Liaison Officer as Coordinator, and one miscellaneous, including Pecan, Genetic Resources and Economics, which is included in the general coordination. Two representatives, one from each supporting institution (FAO and CIHEAM) are also integrated in managing the Network.

The general activities of the FAO-CIHEAM Research Nut Network are proposed, discussed, agreed and planned in the Technical Consultations (participation of representatives of the member countries) and at the Coordination Board meetings (FAO and CIHEAM Officers, Network Coordinator and Sub-network Liaison Officers).

The main activities carried out during the last 20 years have been:

- (i) Organization of specific meetings and workshops.
- (ii) Promotion of R&D activities.
- (iii) Edition of proceedings and reports.
- (iv) Edition of inventories of germplasm and research lines.
- (v) Edition of the NUCIS Newsletter.
- (vi) Organization of two international courses on "Nut Production and Economy".
- (vii) Training grants for young researchers.

The Subnetwork on Stone Pine resumed its activities since the Coordination Board Meeting of the FAO-CIHEAM Network on Nuts, held in 2009. Agropine 2011 has been the first international meeting organized by this Subnetwork in this new period, and it is expected that more events and activities focusing on *Pinus pinea* will be developed in the future under the FAO-CIHEAM Network on Nuts.

Foreword

The pine nut, the edible kernel of the Mediterranean stone pine, *Pinus pinea*, is one of the world's most expensive nuts. Although well known and planted since antiquity, pine nuts are still collected mainly from natural forests in the Mediterranean countries, and only recently has the crop taken the first steps to domestication as an attractive alternative on rainfed farmland in Mediterranean climate areas. Over the last century, the Mediterranean stone pine has experienced a range expansion, especially in the Southern and Eastern Mediterranean Basin, as well as a large increase of planted areas in its countries of origin, both through forest restoration and by farmland afforestation. Today, the Iberian Peninsula accounts for about 75% of the stone pine area in the world, Portugal being the main pine nut producer, followed by Spain, Turkey, Lebanon and Italy. The species performs well on poor soils and needs little husbandry, it is affected by few pests or diseases and withstands adverse climatic conditions such as drought and extreme or late frosts. It is light-demanding and hence has potential as a crop in agro forestry systems in Mediterranean climate zones around the world. Knowledge about stone pine as a crop in grafted plantations is increasing as a result of ongoing research. Plantations on farmland could yield more pine nuts in the future than the natural forests and contribute to rural development and employment of local communities.

Recently, the FAO-CIHEAM Inter-Regional Cooperative Research Network on Nuts restored its sub-network on Stone pine –that had not had any activity since the 1st Symposium on Mediterranean Stone Pine held in Valladolid (Spain) in 2000 –and linked with the former Cooperative Research Network on Stone Pine Silviculture within the framework of FAO Silva Mediterranea (1987-1997). In this context, the international meeting AGROPINE2011 aimed at bringing together the main research groups and potential users in order to gather the current knowledge on Mediterranean stone pine as a nut crop and to analyse its potential and current challenges. The Meeting was organised by the FAO-CIHEAM Network on Nuts, the Mediterranean Agronomic Institute of Zaragoza (IAMZ-CIHEAM), the Spanish National Institute for Agricultural and Food Research and Technology (INIA), the Institute for Agro-food Research and Technologies of Catalonia (IRTA), the Forestry and Forest Industry Services and Promotion Centre (CESEFOR, Centro de Servicios y Promoción Forestal y de su Industria de Castilla y León), and the Forest Sciences Centre of Catalonia (CTFC).

AGROPINE 2011 was held from 17 to 19 November 2011 in Valladolid, located in the northern plateau of central Spain. Valladolid is at the centre of the most important production area of pine nuts in Spain, and stone pine is its most characteristic forest tree. The Meeting was structured into two scientific sessions dealing with management of stone pine for cone production and on genetic improvement, selection and breeding of this species, and was closed by a round table discussion where challenges and opportunities of the pine nut industry and markets were discussed. On the last day a field trip was organized to visit stone pine experimental plots and grafted plantations. Thirty nine scientists, and forest and industry managers, coming from Lebanon, Portugal, Spain, Tunisia and Turkey participated in the meeting, which will hopefully be the first of a series of meetings and activities of the newly restored FAO-CIHEAM Sub-network on Mediterranean Stone Pine.

We acknowledge and thank the support from the organising institutions as well as from the Junta de Castilla y León, the Sustainable Forest Management Research Institute of University of Valladolid-INIA, Piñonsol (Soc. Coop.), the Spanish Agency of International Cooperation for Development (AECID) and the European Regional Development Fund (ERDF).

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Session 1
Management of stone pine
for cone production
in forests and agroforestry

Influence of stand and tree attributes and silviculture on cone and seed productions in forests of *Pinus pinea* L. in northern Tunisia

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Abstract. The present work studied if cone and seed productions were related to tree and stand parameters (age, crown size, stand density and tree social status) in stone pine (*Pinus pinea* L.) stands in Mekna forest (III), Northern Tunisia. The cones were collected in summer 2011 on 10% of the trees sampled in the different trunk diameter classes. They were counted, weighted and classified according to their age (two or three-year-old) and healthy or damaged. Three cones (three-year-old) with different sizes (small, medium and large) per sampled tree were harvested and brought to the laboratory. Seeds were extracted from the cones and their number and total weight per cone were determined. Filled or empty seeds were recorded. The findings showed that cone and seed productions are conditioned by tree size, especially crown volume, age and social status. The number of filled or empty seeds depends on the cone weight. Data showed that cone and consequently seed productions in Mekna III forest were low to extremely low for the considered year. Problems could arise from this deficiency in the future, not only for nut market but also for stand regeneration and species conservation on a long term scale.

Keywords. Cone production – Seed number – Stone pine – Mekna forest (III) – Stand attributes.

Influence des caractéristiques du peuplement, des arbres et des pratiques sylvicoles sur la production de cônes et de graines dans une forêt de Pin pignon au nord de la Tunisie

Résumé. La production de cônes et de graines a été étudiée dans un peuplement de Pin pignon de la forêt de Mekna III au nord de la Tunisie en fonction de certains paramètres des arbres et du peuplement (âge, taille du houppier, densité et situation sociale de l'arbre). Les cônes ont été collectés pendant l'été 2011 sur un échantillon de 10% des arbres selon les différentes classes de diamètre du tronc sur les placettes-échantillons. Ils ont été comptés et classés selon leur âge (deux ou trois ans) ainsi que leur état, sain ou endommagé. Trois cônes de taille différente (petite, moyenne et grande) par arbre ont été pesés et ramenés au laboratoire. Les graines ont été extraites, leur nombre et leur poids ont été déterminés, et les graines pleines et vides ont également été enregistrées. Les résultats montrent que la production de cônes est conditionnée par la dimension, notamment du houppier, l'âge, et l'état social de l'arbre. La quantité de graines, pleines ou vides, dépend du poids du cône. Les données montrent que la production de cônes dans la forêt de Mekna III est très faible pour l'année considérée. Des problèmes pourraient survenir à partir de cette carence dans l'avenir, non seulement pour le marché de pignons destinés à la consommation, mais aussi pour la régénération du peuplement et la conservation de l'espèce à une échelle à plus long terme.

Mots-clés. Production de cônes – Nombre de graines – Pin parasol – Forêt de Mekna III – Caractéristiques dendrométriques.

I – Introduction

The stone pine, *Pinus pinea* L., is a tree species found around the Mediterranean basin. It has been successfully introduced in Tunisia at the beginning of the 20th century along the Mediterranean coast line to consolidate the littoral dunes of Bizerte in the north and along the north east coast in the region of Cap Bon (Hasnaoui, 2000). The success of these first

plantations incited the foresters to use this species to stabilize the littoral dunes of the northwest too. Today *Pinus pinea* covers an area of 21,165 ha (El Khorchani, 2010) and becomes one of the most valuable species in Tunisian reforestation programs, not only for wood production, but also because it is much appreciated for its nuts, widely used in a lot of traditional dishes, such as cakes.

Natural regeneration is difficult to achieve in Tunisia. The yield of cones and the amount of available seeds together with other limiting factors such as livestock and overgrazing often result in the failure of natural regeneration in stone pine stands. Cone yield and seed amount also show great year to year variability, which leads to low masting capacities during long intervals. Therefore a better understanding of the factors controlling cone and seed productions may be key information to provide guidelines for managers to improve both nut production and natural regeneration.

In this study, we report data achieved on the *Pinus pinea* forest of Mekna III, North Tunisia. The aim was to study the effect of tree and stand parameters (tree age, crown volume, stem density, tree social status, pests) as hypothetic key attributes on cone and seed productions.

II – Materials and methods

1. Study area

The study was carried out in the northwest of Tunisia, on coastal dunes of Tabarka (36°57'N, 8°45'E), in the Mekna (III) forest. The climate is Mediterranean with mild winters and long summer dry periods lasting between five and six months. Mean annual temperature is 18.8°C. Average annual rainfall is 934 mm. The area is relatively flat and characterized by a sandy soil with low organic matter, small quantities of sandstone, and a pH above 8. Stone pine constitutes approximately 418 ha of pure or mixed stands.

2. Cone and seed data collection

Two- and three-year-old cones were harvested in late July and 2011. Cones were collected from 97 trees (approximately 10% of trees), sampled in different trunk diameter at breast height (DBH) classes ranging between 10 cm and 65 cm. Tree measurements included DBH, crown height, crown diameter and social status of the tree (i.e. dominated, co-dominant or dominant). The age of the trees was estimated by coring the tree and subsequent ring measurements. Stand tree density and basal area were also calculated.

All cones were manually harvested from each tree, counted, weighted and separated as either healthy or damaged. Three cones (only three-year-old ones) with different sizes (small, medium and large) per tree were chosen and brought to the laboratory. Their length and width were measured (Johnson *et al.*, 2003) with a calliper. Afterwards, the cones were soaked in hot tap water and finally heated in an oven at 60°C for 16 hours to open them. All cones were completely dissected and seeds were extracted. Seeds were counted, weighted and distinguished into filled or empty using the float method (Boydac *et al.*, 2003; Dangasuk and Panetsos, 2004).

III – Results

1. Stands attributes

Pinus pinea stands in Mekna forest (III) presented great differences in their characteristics (Table 1). Tree age was approximately the same in a given stand but varied from 17 to 62 years at the forest scale with a mean value of 36.8. The mean diameter (DBH) was 30 cm and ranged

from 6 to 61 cm, whereas the mean height (H) of the stand was 12.8 m and ranged from 6 to 21 m. Stand density also showed variability, between 140 and 1,820 trees per hectare.

Table 1. Stand characteristics (means \pm SD of the thirty seven sampled plots)

	Mean	Min	Max
Tree diameter(cm)	29.6 \pm 10.3	6	61
Tree height (m)	12.8 \pm 3.7	6	21
Stand density (n/ha)	462 \pm 352	140	1.820
Tree age (years)	36.8 \pm 11.6	17	62

2. Cone production

Pinus pinea exhibited low cone production. Two-year-old cones averaged 5.6 per tree and three-year-old cones showed an average number of 7 per tree (Table 2). Three-year-old cone weight ranged between 60 and 389 g with a mean value of 202 g (Table 2).

Table 2. Cone and seed productions of *Pinus pinea* stands in Mekna forest (III) (mean value \pm standard deviation)

Two-year-old cone number	Three-year-old cone number	Number of filled seed per cone	Percentage of empty seeds (%)	Three year-old cone weight (g)	Total seed weight per cone	Total seed number per cone	Percentage of trees without cones (%)
5.6 \pm 6.0	7.0 \pm 8.1	52.5 \pm 14.4	19.3 \pm 6.2	201.7 \pm 78.4	57.5 \pm 22.5	65.2 \pm 18.4	7.2

Number of two and three-year-old cones was positively and linearly correlated with tree crown volume ($R^2=0.94$ and $R^2=0.90$, respectively; $p<0.0001$; Fig.1). The high crown volume of 613 m³ showed 30 two-year-old cones and 44 three-year-old cones, whereas a small crown volume of 1.5 m³ resulted in an absence of cone.

The age was a worse predictor for cone number than crown volume (for the two- and three-year-old cones $R^2=0.69$ and $R^2=0.84$, respectively; $p<0.0001$).

The number of cones significantly decreased with increasing stand density ($R^2=0.95$; $p<0.0001$). The maximum number of cones found for a density of 140 trees per hectare was 30 and 44 for two- and three-year-old cones, respectively, whereas for a density of 1,820 trees per ha no cone was produced at all. Overall, 7% of trees had no cone.

Crown volume, age and stem density are variables correlated among each other. Results showed a positive correlation between crown volume and age ($r=0.31$; $p<0.01$) and a significant negative correlation between stem density and crown volume ($r=-0.94$, $p<0.0001$). Tree social status had a significant effect on the cone number ($p<0.0001$) with the biggest or dominant trees producing more cones than co-dominated and dominated trees.

3. Seed production

The seed number within the cone was positively and highly correlated with the mean dry weight of the cone ($R^2=99.9\%$, $p<0.0001$; Fig. 2). Larger cones contained more filled seeds. The number of filled seed per cone was 52.5 in mean and ranged from 65.5 to 97.7. Overall mean of empty seeds in this study was 19.3% (Table 2) ranging from 2 to 34%.

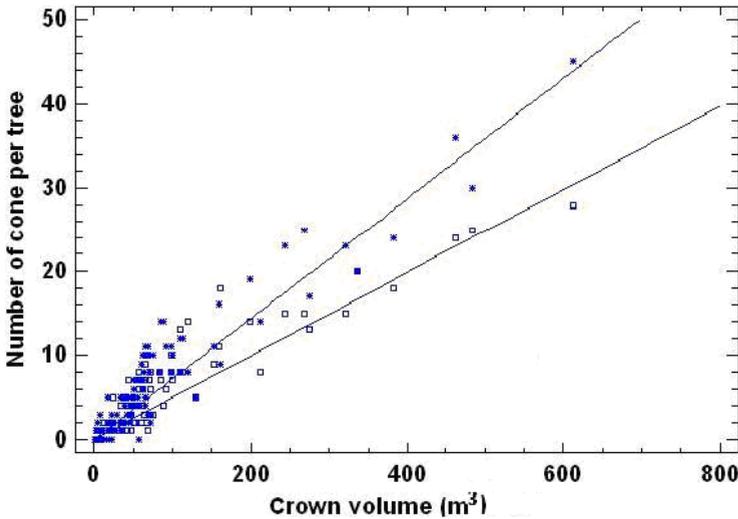


Fig. 1. Cone number relative to crown volume of *Pinus pinea* trees in Mekna (III) forest [□: two-year-old cones, number of two-year-old cones per tree = $(0.0495 \cdot \text{crown volume})$] [*: three-year-old cones, number of three-year-old cones per tree = $(0.0716 \cdot \text{crown volume})$].

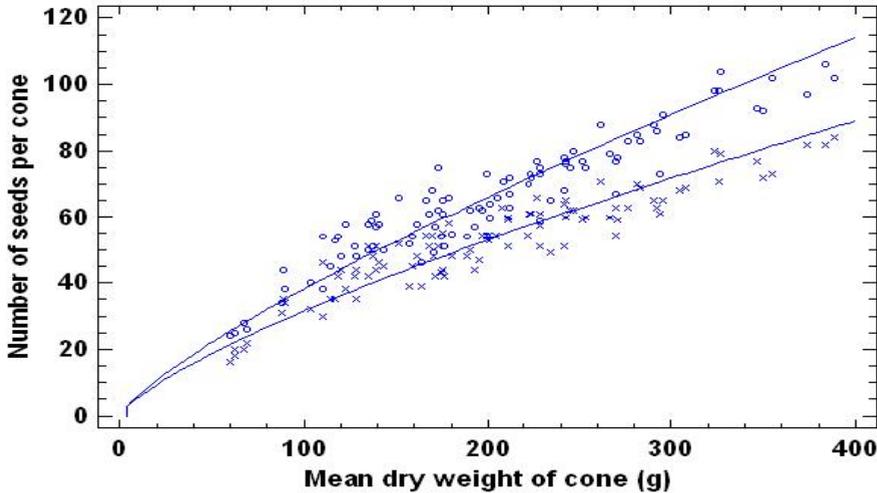


Fig. 2. Number of seeds per three-year-old cone relative to average cone dry weight [○: total seeds, Number of total seeds per cone = $\exp(0.790 \cdot \ln(\text{mean dry weight of cone}))$] [*: only filled seeds, Number of filled seeds per cone = $\exp(0.749 \cdot \ln(\text{mean dry weight of cone}))$].

Our results showed the presence of damages in the sampled two-year-old cones such as a brown colour and small and larger circular wholes. The mean proportion of two-year-old cones attacked by insects was 34.0% in mean for a tree and ranged from 0 to 100%. Infested cones were suspected to have been attacked by *Dioryctria mendacella*, probably *Pissodes validirostris* and Anobiidae (genus *Ernobius*) but we need to confirm this hypothesis (Alain Roques, personal communication).

IV – Discussion

The results highlighted that the biggest trees, with an important crown volume, and being in stands with low tree density produced more cones than smaller trees. Therefore crown volume could be the main factor influencing cone production in the Mediterranean stone pine. At least crown volume seems to have more importance in the determinism of cone production than tree age or tree density. However we have to recognize that we were not able to sample young trees in stands having a low tree density. These results are in the line of those of Gonçalves and Pommerening (2011) who showed a good correlation between crown diameters and coning. Indeed, the largest production was attained when dominant trees were particularly frequent. Calama *et al.* (2008) also stated that the lower the density, the bigger is the number of dominant trees and the higher the site quality the larger is the average cone yield. Calama *et al.* (2011) explained as well the spatial variability of cone production between trees and between plots of stone pine like in our study by the presence of large, dominant trees growing in lightly stocked stands with the highest site quality. They reported for *Pinus pinea* forests on the Spanish Northern Plateau a considerable proportion of trees with no cone production. This result is in contrast to Gonçalves and Pommerening (2011) who reports only three trees in the plots of Alcácer do Sal region (Portugal) without cone. Our results showed 7 trees without cones on a total of 97 samples trees (7.2% trees with no cone).

The seed number per cone was variable and mainly explained by the mean weight of cones. Thus larger and heavier cones contain a higher number or filled seeds. These findings are in accordance with those obtained by Calama and Montero (2005). Correlation between filled seeds and total number of seeds has been identified in our study and in several forest studies since Bramlett and Hutchinson (1964). When the trees are stressed, such as in the case of a high tree density in the stand, their reproductive capacities seem to be reduced, with low cone production, and consequently a lower number of filled seeds and many empty seeds.

Dioryctria mendacella, *Pissodes Validostris* and coleopters (Anobiidae, genus *Ernobius*) seemed to be responsible of many cones attacks but it is necessary to analyse new attacked cones to confirm the presence of these species.

Data showed that cone production by *Pinus pinea* in the Mekna forest was very low and so was consequently the seed production, too. In addition, seeds from the Mekna forest were characterized by a low germination rate (48.5 %, Adili *et al.*, unpublished) in comparison with other studies, such as the site of Strofylia (78 to 98.3%, Ganatsas *et al.*, 2008). Consequently it seems unable to secure species regeneration. In this case appropriate silvicultural practices including pruning and thinning (reduce tree density to increase crown volume) are needed in order to ameliorate cone and seed productions and thus regeneration process.

V – Conclusions

Cone and seed productions of *Pinus pinea* in the Mekna (III) forest, north Tunisia, depend on: (i) the size of the tree and its health; (ii) the number and weight of the cones as factors influencing seed production; and (iii) *Dioryctria mendacella* as a major damaging agent for two-year-old cone. As a result seed and cone productions in the Mekna III forest are low and seem unable to secure species regeneration and nut production.

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Climate factors and their relation regarding cone yield of stone pine (*Pinus pinea* L.) in the Kozak Basin, Turkey

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Abstract. This study aims to reveal the factors that affect cone yield of both natural and planted stone pine (*Pinus pinea* L.) in Kozak Basin, Bergama, İzmir (Turkey). In the basin, productive and non-productive areas have been determined, according to long term observations. Topographical features of the basin, pollination properties and long term precipitation data have been analyzed. In addition, three meteorological stations have been established in places which can represent productive and non-productive areas of the basin. Productive areas have lower relative humidity and higher wind speed than non-productive areas. In non-productive areas the number of days in which the temperature falls below -10°C and relative humidity rates surpass 70% is higher than in productive areas. It has been determined that during pollination period a wind speed higher than 3 m/s is very effective in pollination. Likewise it is understood that pollination also is influenced negatively from precipitation occurring during pollination period, high relative humidity of air and extremely high or low temperatures. In non-productive areas it is determined that there is a temperature difference, reaching to 22°C , between day and night. All these clues show that non-productive areas have a very similar characteristics of a typical "frost hollow (frost pocket)".

Keywords. Kozak basin – Stone pine – Cone yield – Climatic factors – Frost hollow – Frost pocket.

Les facteurs climatiques et leur relation pour la production de cônes du pin pignon (*Pinus pinea* L.) dans le bassin de Kozak en Turquie.

Résumé. Cette étude vise à mettre en lumière les facteurs qui influencent la production de cônes à la fois pour des pins à pignons naturels et plantés (*Pinus pinea* L.) dans le bassin de Kozak, Bergama, İzmir (Turquie). Dans ce bassin, on a mis en évidence des zones productives et non productives selon des observations à long terme. Les caractéristiques topographiques du bassin, les propriétés de pollinisation et les données de précipitations à long terme ont été analysées. De plus, trois stations météorologiques ont été établies dans des lieux pouvant représenter des zones productives et non productives de ce bassin. Les zones productives avaient une humidité relative plus faible et une vitesse du vent plus forte que les zones non productives. Dans les zones non productives par rapport aux zones productives, il y avait un plus grand nombre de jours où la température tombait au-dessous de -10°C et où les taux d'humidité relative dépassaient 70%. Il a été déterminé que pendant la période de pollinisation, une vitesse du vent supérieure à 3 m/s est très efficace pour la pollinisation. De même il est avéré que la pollinisation est également influencée négativement par des précipitations ayant lieu en période de pollinisation, par une forte humidité relative de l'air et par des températures extrêmement fortes ou faibles. Dans les zones non productives on a enregistré une différence de température, atteignant 22°C , entre le jour et la nuit. Toutes ces pistes montrent que les zones non productives ont des caractéristiques très semblables à celles d'une "cuvette de gel" typique (ou poche de gelée)".

Mots-clés. Bassin de Kozak – Pin pignon – Production de cônes – Facteurs climatiques – Cuvette de gel – Poche de gelée.

I – Introduction

One-third of the total stone pine area in Turkey (16,000/50,000 ha) and two thirds of the national pine nut production (800 to 1,200 t/yr) are located in the Kozak basin, in the Aegean Bergama District near İzmir, a bowl shaped basin with granite bedrock. In spite of the importance of the

area for the Turkish pine nut production, stone pine growers in Kozak Basin have been complaining about low and irregular yields and extreme conelet loss. But most previous studies on the basin were about ecological adaptation and growth of stone pine (Fırat, 1943; Akgül and Yılmaz, 1991; Kılıcı *et al.*, 2000; Çukur *et al.*, 2005), whereas factors that affect cone yield have not been studied yet. In recent years, increased conelet losses have been observed in stone pine, and a study to uncover relations among cone losses and biotic factors and nutrition has been conducted by Aegean Forestry Research Institute.

According to the study, almost all of the cone losses happen in the first year. Almost all new born conelets fall off in July and August. According to the statistical analysis, there is no relation between amount of new born conelets and altitude, but there is a negative correlation between altitude and conelet losses. When altitude increases, conelet losses decrease (Özçankaya *et al.*, 2010). In the study, also diseases and pests causing cone losses have been determined, however, none of them have been found effective on conelet losses. The areas with 5-10 ripe cones per tree were accepted as unproductive, while those with more than 50 ripe cones per tree were accepted as productive. Besides, Özçankaya *et al.* (2010) reported that in a trial area only one new conelet out of 111 conelets survived at the end of September. This effect might be due to insufficient pollination caused by some climatic conditions. Roques (1977) also reports that cone losses due to various abiotic factors causing infertility. In this context, the aim of the present work was to study relations between location and climatic features of the basin and pollination success.

II – Material and methods

Yield data have been gathered from local growers and cooperatives. Productive and non-productive areas have been also determined according to the information given by owners. In first place, areas with low and high yield within the basin have been marked on a topographic map, finding that areas with high cone yield are above certain altitude (500 m a.s.l.) (Özçankaya *et al.*, 2010). Afterwards, meteorological station locations in these areas and climatic factors have been evaluated. In the study, 1:25000 scale maps of DSI (State Directorate for Water Affairs) were utilized, as well as long term meteorological data of DMI (State Directorate for Meteorology) and data obtained from portable weather stations established in the basin.

Historic climate records showed an average precipitation of 939 mm in the village Yukarıbey [500 m a.s.l., 1963-1998] and 741 mm at Güneşli Station [700 m a.s.l., 1980-1994]. Although both station are located in the same basin and the distance between them is less than 10 km, there is a precipitation difference of nearly 200 mm, though the altitude difference between both is 200 m. The reason might be the prevalent north winds that leave more precipitation at windward mountains slopes than at lee (Anonymous, 2010). Occasional fog occurs in the basin in winter and autumn (Fig. 1).



Fig. 1. Foggy Landscape of Upper Kozak Basin.

For our study, three meteorological stations have been established in places which can represent the basin and its productive and non-productive areas: (i) Karaveliler village, at 587 m a.s.l. (in upper Kozak Basin, with high yield and altitude); (ii) Pompa, at 430 m (in upper Kozak Basin, with low yield and medium altitude); and (iii) Demircidere village, at 230 m (in lower Kozak Basin, low yield and altitude) (Fig. 2).

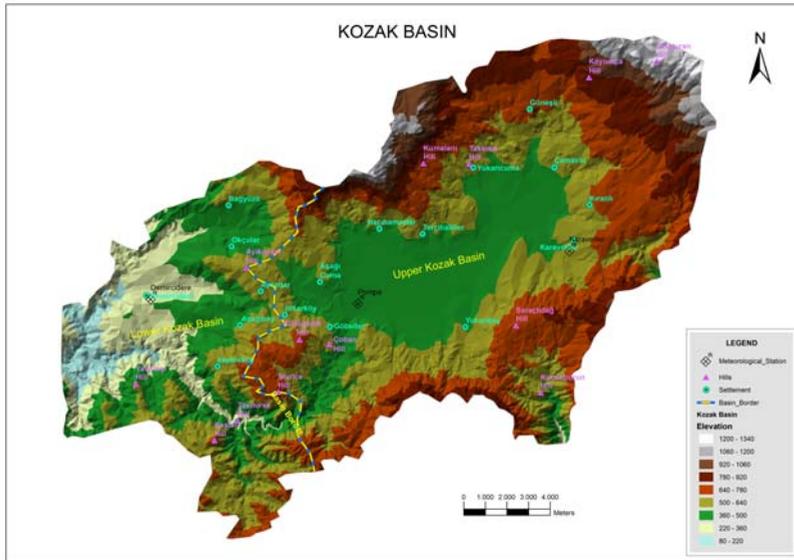


Fig. 2. Kozak Basin Digital Elevation Model (DEM) and localization of the three weather stations (Karaveliler, Pompa and Demircidere).

As the crow flies, distances between stations are almost equal (about 10 km). Each weather station registered hourly humidity, temperature and wind speed parameters during 2009 and 2010. Phenology and cone survival was monitored in both years, in order to evaluate recorded weather data during pollination period such as temperature, relative humidity and wind speed.

III – Results

Observed pollination period in the basin varies from mid-May to early June, according to the observations for many years. It depends on altitude: between May 19 and 31 in the Upper Kozak Basin village Karaveliler, from May 28 to June 3 around Pompa, 12th and 20th may in Demircidere Village (Table 1). There were large differences between day and night temperatures in May in both years, 14°C in Karaveliler, 22°C around Pompa and 16°C in Demircidere. Average humidity was between 47-60% in Karaveliler, 77-85% in Pompa and 59% in Demircidere, and average wind speeds 1.45-0.94 m/s in Karaveliler and Pompa, 0.75-0.67 m/s in Demircidere (Table 1).

Although in both years average and maximum temperatures were similar among sites, minimum temperatures differed clearly. Temperatures below 10°C occurred only once in Karaveliler in 2009, none in 2010, and six times (hours) in Demircidere in 2009, but 77 and 95 hours in Pompa in the two years.

On the other hand, during pollination period, the lowest average relative humidity was in Karaveliler, followed by Demircidere and Pompa. Relative humidity over 70% occurred in

2009 only during 32 hours in Karaveliler, but for 150 in Demircidere and 294 hours in Pompa, and in 2010, 172 hours in Karaveliler versus 360 in Pompa. The average wind speeds during pollination period was highest in Karaveliler and lowest in Demircidere. Wind speeds faster than 3 m/s, considered effective for pollination, were never observed in Demircidere, in Pompa once, but 48 times in Karaveliler during the studied period in 2009, 20 times in Karaveliler in 2010, when in Pompa only 8 hours passed this threshold.

Table 1. Climate data recorded during pollination period at the three meteorological stations

Locality	Pollination period	Year	Temperature (°C)			Relative humidity (%)	Avg. wind speed (m/s)	Wind >3m/sn (hours)	Humidity >70% (hours)	Temp. <10°C (hours)
			Avg.	Max.	Min.					
Karaveliler (587 m) Prod.	19-31 May	2009	20.0	32.1	10.0	47%	1,45	48	32	1
		2010	18.5	32.4	7.4	60%	0,94	20	172	0
Pompa (430 m) Unp.	28 May-3 June	2009	17.5	29.8	1.7	77%	0,75	1	294	77
		2010	16.3	30.8	-0.1	85%	0,67	8	360	95
Demircidere (230 m) Unp.	12-20 May	2009	20.6	31.4	8.3	59%	0,39	0	150	6

IV – Discussion and conclusions

Pollination period varied between 12th May and 3rd June due to altitude in both sub-basins. The observed difference among weather station allows relating cone setting with climate data. Pollination happens by means of wind in pine species (Anşın, 1988), and wind speed has an important role in burst of pollen strobili and airborne pollen transport to female conelets. Weak winds are inefficient not only for pollen shedding but also for its transfer; and a wind speed minimum of 3 m/s is required for moving the male branchlets swinging back and forth continuously (Öztürk and Seçmen, 2004). Moreover, relative humidity which has an inverse ratio with wind speed also affects negatively pollen shedding. Thus it seems that there is not sufficient wind speed and dry air conditions in Demircidere and Pompa, although all trees have both male and female flowers.

On the other hand, climate parameters recorded during pollination (19-31 May) period of 2010 substantially are different from that of 2009. Number of hours with wind speed (3 m/s or above) needed for pollination in 2010 were quite lower than in 2009 at Karaveliler, and also mean temperature and wind speed were lower, but precipitation was higher. These air conditions are considered to have negatively affected the pollination. Pollen transfer could be affected due to wind speed and excess humidity, cold and humid air might keep closed male and female flowers (Adkins *et al.*, 2005). The negative impact of these climatic factors increased conelet losses in 2010. More loss than ordinary was also observed in Karaveliler village, located in Upper Kozak basin where conelet loss is minimum in normal years. That year, unusual conelet losses happened and it even continued in September while normally occurring mainly in June, July and August. It is also possible that unusual humid climatic conditions in 2010 might have triggered some fungal diseases.

Regarding wind speeds over 3 m/s during pollination period that are supposed to be very effective on pollination, only the upper basin sections with altitude over 500 m have enough wind, but lower basin does not. Again precipitation, high relative humidity and extreme temperatures have negative impacts on pollination during pollination period.

Moreover, stone pine stands in the Lower Kozak Basin are dense and overstocked, therefore there might not be sufficient air circulation for pollination, and interventions such as thinning and pruning are necessary. The sections between 430-500 m at the plain in Upper Kozak Basin

show characteristics of a frost pit. Therefore it is more appropriate to sustain traditional agricultural activities here rather than stone pine plantations for cone production.

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Effects of nutrients on cone losses of stone pine (*Pinus pinea* L.) in Kozak Basin

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Abstract. Kozak Basin is located in Bergama District in Izmir Province. The basin is a bowl-shaped stone pine forest area, with a size of 16,000 hectares, which occupies one-third of the total stone pine area in Turkey. Almost all the forest area in the Basin is private. In recent years, cone losses of stone pine have increased. For this reason, in this study 8 different areas were examined to determine the relations between cone losses and nutrients over the 2005-2008 period. Almost all of cone losses occurred in the new conelets according to the results of cone counting in the sampling area. Therefore, evaluations were conducted based on this period. Significant differences in cone losses were found between years and localities. To determine the relationship between cone losses and nutrients, one-, two- and three-year old needles were taken from the sample trees and their size and weight were measured, then the samples were analysed in terms of micro- and macro nutrients. Soil samples were taken beneath the same sample trees and analysed. By examining the relationship between cone losses and nutrients in the needles, a negative correlation between cone losses and nitrogen (N), phosphorus (P), calcium (Ca) and manganese (Mn) could be determined. In other words, when these nutrients diminished, cone losses increased. It is considered that among these elements, especially P and Ca combined with drought could be a factor in cone losses.

Keywords. Stone pine – Cone loss – Cone yield – Nutrient.

Effet des nutriments sur les pertes en cônes chez le pin pignon (*Pinus pinea* L.) dans le bassin de Kozak

Résumé. Le bassin de Kozak se trouve situé dans la zone de Bergama, province d'Izmir. Ce bassin est une zone forestière de pins pignons en forme de cuvette, d'une superficie de 16000 hectares, qui représente un tiers de la surface totale de pin pignon de la Turquie. Presque toute la surface forestière de ce bassin est de propriété privée. Dernièrement, la perte de cônes chez les pins pignons s'est accrue. C'est pour cette raison que dans le cadre de cette étude, 8 zones différentes ont été examinées afin de déterminer le rapport entre les pertes de cônes et les nutriments disponibles sur 2005-2008. Pratiquement toutes les pertes de cônes avaient lieu sur les jeunes cônes selon les résultats du dénombrement dans la surface d'échantillonnage. Donc, des évaluations ont été menées basées sur cette période. Des différences significatives de perte de cônes furent trouvées entre années et localités. Afin de déterminer le rapport entre perte de cônes et nutriments, des aiguilles de pin d'une, deux et trois années d'âge furent prélevées sur les arbres échantillonnés pour mesurer leur taille et leur poids, et pour les analyser ensuite concernant les micro et macronutriments. Des échantillons de sol furent prélevés sous ces mêmes arbres échantillonnés, et analysés. D'après l'examen du rapport entre pertes de cônes et nutriments dans les aiguilles de pin, une corrélation négative a été déterminée entre pertes de cônes et azote (N), phosphore (P), calcium (Ca) et manganèse (Mn). Autrement dit, lorsque ces nutriments diminuaient, les pertes de cônes augmentaient. On considère que parmi ces éléments, ce sont spécialement le P et le Ca qui, combinés à la sécheresse, pourraient être un facteur intervenant dans la perte de cônes.

Mots-clés. Pin pignon – Perte de cônes – Production de cônes – Nutriments.

I – Introduction

Kozak Basin is located in Bergama District in Izmir Province. The basin is a bowl-shaped stone pine forest area, with a size of 16,000 hectares, which occupies one-third of the total stone pine

area in Turkey. Almost all the forest area in the Basin is private, some belongs to the Government. There seems to be a decrease in cone productivity in various parts of the Basin since 2005. Therefore, this study aims to identify the effects of nutrients on conelet losses of stone pine in Kozak Basin in Izmir Province. For this purpose, relationships were investigated between nutrition status of trees in the same watershed from different localities and conelet losses. Thus, soil properties and nutrient contents of sampling areas and nutrient contents of needles were analysed and determined statistically.

Up to now, the Basin has been subjected to several studies focusing on different aspects. Çukur (1994) examined natural potential of growth conditions of stone pine communities in Aegean region and their contribution to the economy of the region and the country. Bilgin and Ay (1997) investigated pine nut industry in Bergama -Kozak and Aydın- Koçarlı regions in Aegean region and its contribution to the economy. Kılıcı *et al.* (2000) studied the factors affecting stone pine growth in the west of Anatolia –which also includes Kozak region– and revealed the ecological characteristics of the natural and planted stone pine areas, compared them and gave suggestions for new plantation areas. Another study, which is currently continued by Kılıcı and his colleagues, will give the effect of fertilization on cone productivity in a stone pine plantation area bearing low yield in Kozak region at the end of 2012. Calama *et al.* (2007) analysed the effect of mineral fertilization on cone production and size, which are closely related to nut yield and quality in south west Spain and compared the effect of different doses of lime superphosphate, dolomite and potassium.

II – Materials and methods

The material of this study was composed of one-, two- and three-year old cones and needles and soil samples in stone pine stands in Kozak Basin.

In this study, the relationships between nutrients and conelet losses in eight different sampling areas in Kozak province were determined in 2005-2008. Plots placed in Kozak region on granite bedrock were determined from two altitudes (500 m and above, 500 m and below), two wind conditions (open to sea wind and not) and number of cones on tree (few and abundant). In total, eight sampling areas were chosen with these factors (Table 1). Five trees and two branches on each tree –preferably middle parts of the trees– a total of 80 branches were chosen and marked. All branches were determined twice a month during the vegetation period, once a month out of the vegetation period, and then one-year old cones (conelets), two- and three-year old cones were counted. In the top 1/3 part of the crown, and from the branches in the sunny side, one-, two- and three-year old needles from each tree were collected for the analysis. In each sample site soil samples representing the area were taken for the determination of the physical and chemical properties and macro-micro elements of soil regarding soil horizons.

Table 1. Locations and some properties of sampling areas

Field No.	Altitude (m)	Sea wind	Cone existence	Average rainfall (mm) [†]
1	718	-	+	939
2	577	-	-	743
3	688	+	+	939
4	380	+	-	743
5	225	+	-	743
6	245	-	-	743
7	523	+	+	743
8	450	-	+	939

[†]Source DSI (1963-1998).

III – Results

1. Soil properties

Bedrock material of all the sampling areas is granite. Soil properties are given in Table 2 as averages of horizons. This bedrock creates coarse textured soils when it is fragmented. Soils of all sampling areas have coarse texture. As is seen in Table 2 soils are sand, loamy sand and sandy loam. Soil reaction (pH) of all areas is between 6.07 and 6.67. Therefore, they are slightly acidic and neutral. Additionally, soils of all areas are salt- and lime- free. Organic material amounts of top soils in the sampling areas are between 1.7 and 8.1%. All areas have high and very high organic matter content. There are differences between sampling areas in terms of the nutrients.

2. Needle properties

Figure 1 shows the needle lengths determined in the study area. The lengths of the needles collected from the sampling areas were between 9.56 and 14.95 cm. Our observations showed that there was a lack of water in the sampling areas 2, 4, 5, and 6 (there is no evidence of water in those plots such as a stream or spring) in the summer months (though there is no meteorological station around the study area except for the DSI rainfall station). Therefore, the needle lengths in those sampling areas were shorter and they were sparse. Almost all of the three-year old needles fell on the ground. In the sampling areas 1, 3, 7 and 8 there was no lack of water (there are some springs pouring even in summertime) and the needle lengths were longer in those areas.

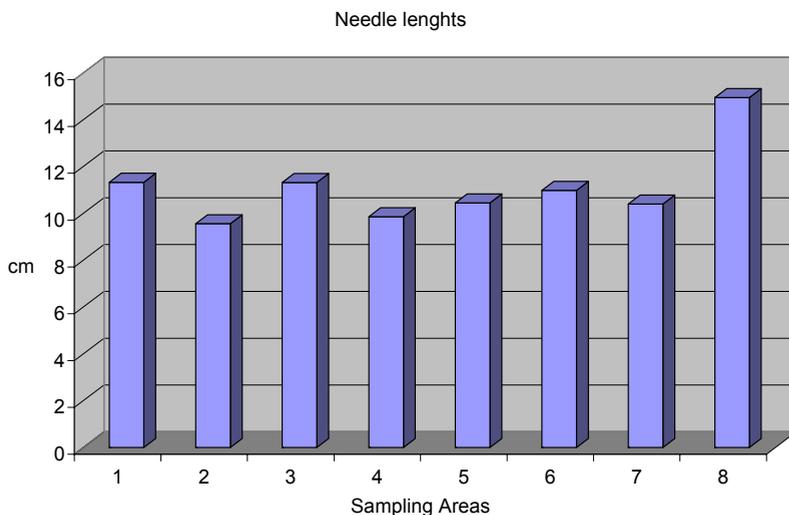


Fig. 1. Mean needle lengths (cm) determined in the sampling areas.

Fresh weights of the needles were 10.27-17.87 g (g/100 needles), and dry weights of them were 4.53-7.79 g (g/100 needles) (Fig. 2).

Table 2. Physical and chemical properties of soils in the study area

Plot No.	Sand (%)	Clay (%)	Silt (%)	Texture (USDA)	pH (1:2.5 water)	N (%)	P (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)	Na (ppm)	Fe (ppm)	Cu (ppm)	Zn (ppm)	Mn (ppm)
1	73.97	6.77	19.25	SL	6.12	0.08	8.8	75	2166	448	9	35	0.5	0.7	26.7
2	71.92	8.13	19.95	SL	6.11	0.12	42.6	182	982	491	10	27	0.8	1.9	25.3
3	75.25	7.13	17.61	LS	6.07	0.11	29.5	110	876	250	9	19	0.7	3.5	20.2
4	80.92	4.80	16.28	LS	6.43	0.15	15.9	110	1490	355	6	10	0.5	4.8	29.5
5	82.25	4.80	12.95	LS	6.54	0.08	8.9	92	1153	295	6	6	0.8	3.5	47.9
6	77.59	7.80	14.61	LS	6.69	0.06	7.8	90	1123	378	9	6	0.8	1.6	25.4
7	78.59	5.80	14.95	LS	6.41	0.06	5.3	55	1166	348	20	15	0.3	1.0	17.6
8	88.59	2.47	8.95	S	6.19	0.13	9.0	46	703	166	9	40	0.7	2.3	12.9

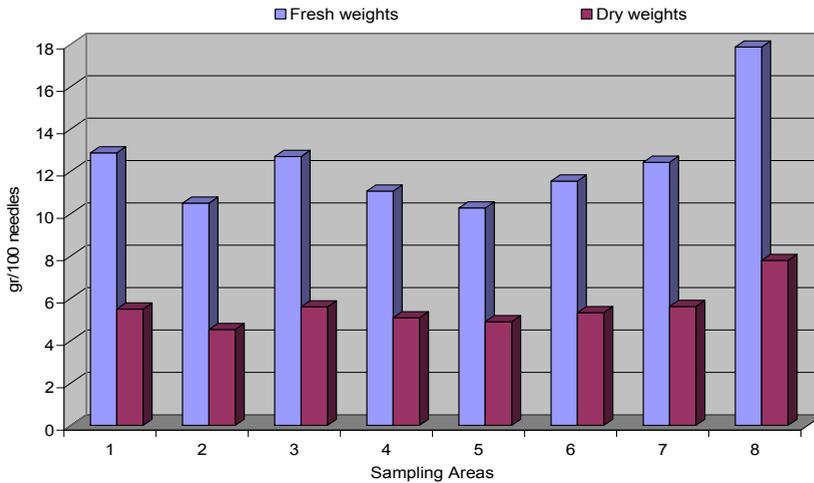


Fig. 2. Mean needle fresh and dry weights determined in the sampling areas (g/100 needles).

3. Cone losses

Figure 3 shows one-year old cone losses in the sampling areas in 2005-2008. In all sampling areas, most of the cone losses were observed in one-year old cones. Therefore, these conelet losses were taken into account in the evaluations. In the sampling areas 2, 4, 5 and 6 most of the cone losses happened in one-year olds. In some areas the trees had normal amount of conelets at the beginning of the season, but especially in July and August they lost almost all of their conelets. For example, in the 1st sampling area, 65 conelets were counted at the beginning of the season in 2005. At the end of the season, 62 of them were alive. But in the 4th sampling area, 111 conelet were counted at the beginning of the season. By the end of July, only half of this amount was alive. Only one conelet stayed alive at the end of the summer season. The sampling areas 1, 3, 7 and 8 had higher living percentage of conelets than the other sampling areas.

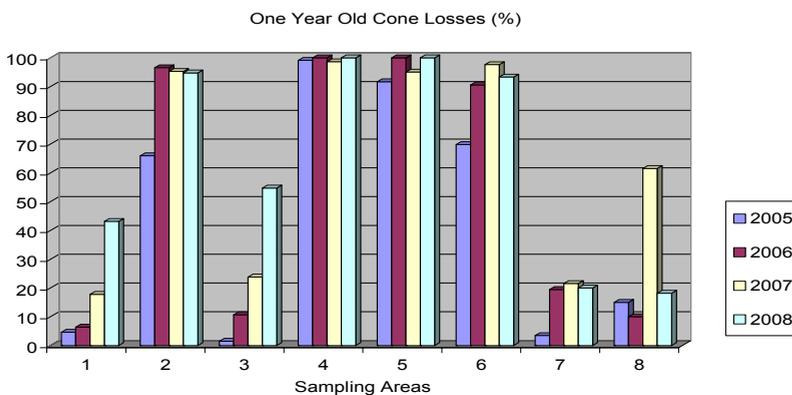


Fig. 3. One-year old cone losses in the sampling areas according to the year.

According to the statistical assessments there was a negative correlation between cone losses and altitude, needle lengths, needle fresh/dry weights (Table 3) and some nutrients in the

needles (Table 4). However there no correlation was found between conelet formation or needle size and altitude.

Table 3. Correlation of cone losses and needle lengths, needle fresh/dry weights and altitude

	Length of needle	Needle fresh weight	Needle dry weight	No. of conelet	Conelet loss	Percentage of loss	Altitude
Length of needle		0.885**	0.863**	-0.126ns	-0.273ns	-0.361*	0.044ns
Needle fresh weight	0.885**		0.917**	-0.133ns	-0.429**	-0.506**	0.241ns
Needle dry weight	0.863**	0.917**		-0.104ns	-0.295ns	-0.364*	0.051ns
No. of conelet	-0.126ns	-0.133ns	-0.104ns		0.588**	0.090ns	0.000ns
Loss of conelet	-0.273ns	-0.429**	-0.295ns	0.588**		0.691**	-0.550**
Percentage of loss	-0.361*	-0.506**	-0.364*	0.090ns	0.691**		-0.776**
Altitude	0.044ns	0.241ns	0.051ns	0.000ns	-0.550**	-0.776**	

V – Discussion

The sampling areas were located on granite bedrock and lime-free soil. Almost all of the cone losses occurred in one year-old cones with respect to the cone counts in the sampling areas. Therefore, this period was taken into account for the evaluations. There were significant differences determined in cone losses between years and regions (Fig. 3). Except for the sampling area 1, the rest of the areas have low Ca content in the soil (Table 2). It was determined that cone losses increased while N, P, Ca and Mn amounts of the needles decreased (Table 4). Among these elements, especially P and Ca displayed more significant results during the correlation analysis. It is known that these elements have positive effects on fruit and seed formation (Kaçar, 1977; Özbek *et al.*, 1984). Calama *et al.* (2007) showed that best cone crops are attained with treatments adding larger amounts of phosphorous, potassium chloride and dolomite, leading to average crops per stone pine tree over 3 times larger than control in the sandy areas having low nutrients and lime content in Spain. The differences among Ca amounts in the sampling areas might be resulted from the location of the sampling fields and the existence of water leakage.

Correlation analysis was applied to question the relationship between needle lengths, needle fresh/dry weights and cone losses, and a negative correlation was detected in between them (Table 3). Cone losses increased while needle length and needle fresh/dry weight decreased. The reason of this situation might be water deficiency. Thus, in plot 8 there is no water deficiency because of topographical conditions (it is placed in flat area in lower altitude); although there is lower soil nutrient content in this area than in other plots, water sufficiency reduces cone losses.

There were also field comparisons conducted in relation to the cone losses (Fig. 3). In some fields, as field no 2 and 4, cone losses were abundant although there were much more nutrients in the soil. However it was determined that the trees did not take the nutrients sufficiently according to the data such as needle lengths, needle weights and needle nutrient content. Water deficiency in the soil might be the reason for this situation. Since it was found that there are soluble nutrients like K, Ca, Mg, Na, PO₄ and NO₃ in gravitation water in the other parts of Kozak region (Kılıcı *et al.*, 2000).

Table 4. Correlation of cone losses and (macro and micro) nutrient contents in needles

	N	P	K	Ca	Mg	Na	Fe	Cu	Zn	Mn	Conelet counted	Conelet loss	Conelet loss (%)
N		0.68 **	0.56 *	0.40 **	0.45 **	0.73 **	0.80 **	0.07 <i>ns</i>	0.06 <i>ns</i>	-0.02 <i>ns</i>	-0.08 <i>ns</i>	-0.26 <i>ns</i>	-0.37 *
P	0.68 **		0.56 **	0.39 *	0.24 <i>ns</i>	0.31 <i>ns</i>	0.61 **	0.09 <i>ns</i>	0.01 <i>ns</i>	0.28 <i>ns</i>	-0.19 <i>ns</i>	-0.34 *	-0.34 *
K	0.56 **	0.56 **		0.38 *	0.09 <i>ns</i>	0.35 *	0.53 **	0.20 <i>ns</i>	0.06 <i>ns</i>	0.24 <i>ns</i>	0.09 <i>ns</i>	-0.21 <i>ns</i>	-0.31 <i>ns</i>
Ca	0.40 **	0.39 *	0.38 *		0.39 *	0.15 <i>ns</i>	0.19 <i>ns</i>	0.19 <i>ns</i>	0.40 *	0.55 **	0.08 <i>ns</i>	-0.18 <i>ns</i>	-0.47 **
Mg	0.50 **	0.23 <i>ns</i>	0.09 <i>ns</i>	0.39 *		0.35 *	0.32 *	-0.20 <i>ns</i>	0.21 <i>ns</i>	0.04 <i>ns</i>	0.23 <i>ns</i>	0.23 <i>ns</i>	0.05 <i>ns</i>
Na	0.73 **	0.31 <i>ns</i>	0.35 *	0.15 <i>ns</i>	0.35 *		0.61 **	-0.01 <i>ns</i>	0.10 <i>ns</i>	-0.29 <i>ns</i>	-0.05 <i>ns</i>	-0.03 <i>ns</i>	-0.18 <i>ns</i>
Fe	0.80 **	0.61 **	0.53 **	0.19 <i>ns</i>	0.32 *	0.61 **		0.06 <i>ns</i>	-0.20 <i>ns</i>	-0.15 <i>ns</i>	-0.06 <i>ns</i>	-0.23 <i>ns</i>	-0.27 <i>ns</i>
Cu	0.07 <i>ns</i>	0.09 <i>ns</i>	0.20 <i>ns</i>	0.19 <i>ns</i>	-0.20 <i>ns</i>	-0.01 <i>ns</i>	0.06 <i>ns</i>		0.35 *	0.18 <i>ns</i>	-0.19 <i>ns</i>	-0.16 <i>ns</i>	-0.04 <i>ns</i>
Zn	0.06 <i>ns</i>	0.01 <i>ns</i>	0.06 <i>ns</i>	0.405 *	0.21 <i>ns</i>	0.10 <i>ns</i>	-0.20 <i>ns</i>	0.35 *		0.42 **	0.08 <i>ns</i>	0.02 <i>ns</i>	-0.13 <i>ns</i>
Mn	-0.02 <i>ns</i>	0.28 <i>ns</i>	0.24 <i>ns</i>	0.55 **	0.04 <i>ns</i>	-0.29 <i>ns</i>	-0.15 <i>ns</i>	0.18 <i>ns</i>	0.42**		-0.14 <i>ns</i>	-0.41 **	-0.48 **
Conelet counted	-0.08 <i>ns</i>	-0.19 <i>ns</i>	0.09 <i>ns</i>	0.08 <i>ns</i>	0.23 <i>ns</i>	-0.05 <i>ns</i>	-0.06 <i>ns</i>	-0.19 <i>ns</i>	0.08 <i>ns</i>	-0.14 <i>ns</i>		0.56 **	0.09 <i>ns</i>
Conelet loss	-0.26 <i>ns</i>	-0.34 *	-0.21 <i>ns</i>	-0.18 <i>ns</i>	0.23 <i>ns</i>	-0.037 <i>ns</i>	-0.23 <i>ns</i>	-0.16 <i>ns</i>	0.02 <i>ns</i>	-0.41 **	0.60 **		0.71 **
Conelet loss (%)	-0.37 *	-0.335 *	-0.310 <i>ns</i>	-0.471 **	0.052 <i>ns</i>	-0.176 <i>ns</i>	-0.269 <i>ns</i>	-0.041 <i>ns</i>	-0.128 <i>ns</i>	-0.477 **	0.088 <i>ns</i>	0.706 **	

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Effects of pests and diseases on stone pine (*Pinus pinea* L.) conelet losses in Kozak catchment area

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Abstract. Kozak catchment area is in Bergama District in İzmir, a bowl shaped area with 16,000 hectare stone pine forest which forms one-third of the total stone pine area in Turkey. Almost all the forest area is private. In recent years, conelet losses of stone pine have been increased. To investigate the relation between the losses and pest-disease effects, 8 different sample areas were examined during 2005–2008. The results showed that almost all cone losses occurred in new conelets. In the cone losses, significant differences were found between years and sample areas. But neither a pest nor a disease was found as the effect of the high losses. The most abundant insect species on two and three aged cones was *Dioryctria pineae* Staudinger (Lep., Pyralidae). Damage rate of this pest was 3% in some areas. Other pests were identified as *Ernobius pini* (Col., Anobiidae), *Camptomyia pinicola* (Dipt. Cecidomyiidae), *Cicada* sp. (Hom., Cicadidae), *Apterygothrips priesneri* zur Strassen (Thys., Phlaeothripidae) and *Neohydatothrips gracilicornis* (Williams) (Thys., Thripidae). And some pests and diseases which caused indirect conelet losses –making some damages on shoots– were identified as *Tomicus* sp. (Col., Curculionidae), *Sordaria* sp. and *Pestalotiopsis* sp..

Keywords. Stone pine – Cone losses – Pest – Disease.

Effet des maladies et ravageurs sur les pertes en jeunes cônes de pin pignon (*Pinus pinea* L.) dans le bassin hydrographique de Kozak

Résumé. Le bassin hydrographique de Kozak se trouve dans la zone de Bergama, Izmir, une superficie en forme de cuvette de 16 000 hectares de forêts de pins pignons qui constitue un tiers de la surface totale de pins pignons de la Turquie. Presque toute la surface forestière est de propriété privée. Dernièrement, les pertes de jeunes cônes de pins pignons se sont accrues. Pour étudier le rapport entre ces pertes et les effets des ravageurs et maladies, 8 différentes parcelles d'échantillonnage ont été examinées sur les années 2005–2008. Les résultats ont montré que presque toutes ces pertes avaient lieu sur les jeunes cônes. Des différences significatives quant aux pertes de cônes ont été trouvées entre années et parcelles d'échantillonnage. Mais aucune maladie ni ravageur ne s'est avéré être la cause de ces fortes pertes. L'espèce la plus abondante d'insectes sur les cônes de deux ou trois ans d'âge était *Dioryctria pineae* Staudinger (Lep., Pyralidae). Le taux de dommages de ce ravageur était de 3 % dans certaines zones. D'autres ravageurs ont été identifiés comme étant *Ernobius pini* (Col., Anobiidae), *Camptomyia pinicola* (Dipt. Cecidomyiidae), *Cicada* sp. (Hom., Cicadidae), *Apterygothrips priesneri* zur Strassen (Thys., Phlaeothripidae) et *Neohydatothrips gracilicornis* (Williams) (Thys., Thripidae). Et certains ravageurs et maladies qui causaient des pertes indirectes de jeunes cônes – provoquant quelques dégâts sur les bourgeons – furent identifiés comme *Tomicus* sp. (Col., Curculionidae), *Sordaria* sp. et *Pestalotiopsis* sp..

Mots-clés. Pin pignon – Perte de cônes – Ravageur – Maladie.

I – Introduction

Kozak basin is in Bergama District near İzmir, a bowl shaped area with 16,000 hectare stone pine forest which forms one-third of the total stone pine area in Turkey. Almost all the forest area is private. In recent years, conelet losses of stone pine have been increased.

Previous studies about pest and disease effects on *Pinus pinea* L. cones exist. Roques (1983) investigated some cone pests according to the development stages of 7 pine species (*Pinus sylvestris*, *P. uncinata*, *P. nigra*, *P. halepensis*, *P. pinaster*, *P. pinea* and *P. cembra*) cones. Also,

Kanat (2001) in Kahramanmaraş-Önsen; Can and Özçankaya (2004) in Aegean Region and Mutke *et al.* (2007) in Spain investigated some stone pine cone pests. But in recent years, conelet losses of stone pine have increased in Kozak. In this study, the relationship between conelet losses and pest-disease effects was investigated.

II – Materials and methods

The materials of the study were consisted of one, two and three aged cones (one aged cones: 0-12 months; two aged cones: 12-24 months; three aged cones: older than 24 months) collected from the sample areas and biotic agents (insects and diseases) which were obtained from the damaged cones.

In Kozak catchment area, 8 different sample areas were chosen according to: 2 different altitudes (higher and lower than 500 m), two wind conditions (open the sea wind and not) and the abundance of conelets (high or low) (Table 1). There were no regular data about the amount of conelets so the declarations of the land owners were considered as a basis for classifying.

Table 1. Some properties of the sample areas

Sample area no.	Altitude (m)	Exposed to sea wind	Abundance of cones	Texture	pH
1	710	-	High	SL (sandy loam)	6.12
2	590	-	Low	SL (sandy loam)	6.11
3	720	+	High	LS (loamy sand)	6.07
4	380	+	Low	LS (loamy sand)	6.43
5	230	+	Low	LS (loamy sand)	6.54
6	270	-	High	LS (loamy sand)	6.69
7	550	+	Low	LS (loamy sand)	6.41
8	480	-	High	S (sand)	6.19

In each sample area, to control and count one, two and three aged cones, five trees were chosen, from which two branches - preferably located at the middle of the crown- were marked. From 2005 to 2008, cone observations were made twice a month during the vegetation season and once a month in the rest of the year. Damaged or dried cones were taken to the laboratory for the detailed examinations. Cones were counted by the same two experienced persons.

Brought insect samples at the immature stages were observed and their biological data were recorded at the laboratory conditions. Obtained adults were identified by the specialists.

After bringing the diseased plant samples into the laboratory, the infected tissues along with adjacent small unaffected tissue were cut into small pieces (2-5 mm squares). They were transferred to sterile glass-beaker containing 0.5 % sodium hypochlorite solution used for surface sterilization of plant tissues for a period of 30-60 s. The tissue pieces were washed twice by transferring briefly to sterile distilled water. The pieces then were dried on sterile filter paper. The sterilized pieces were aseptically transferred to petridishes containing potato dextrose agar (PDA) supplemented with streptomycin sulfate, at the rate of three to five pieces of tissues per petri plate and incubated at room temperatures (24-25°C) that may favor the pathogen development.

III – Results

The results showed that almost all cone losses occurred in new conelets. Therefore, evaluations were based on this period. In the cone losses, statistically significant differences were found between years and sample areas (0.1 %).

There were statistically significant differences (0.1 %) between the sample areas according to the living percentages of cones during two periods that recorded from the first occurrence of cones to the reaping time (the first period: 2005-2007; the second period: 2006-2008). 1st, 3rd, 7th and 8th sample areas had a higher living percentages of cones than the other areas (Fig. 1). Determinated insect pests and diseases are mentioned according to the rate of damage (Table 2):

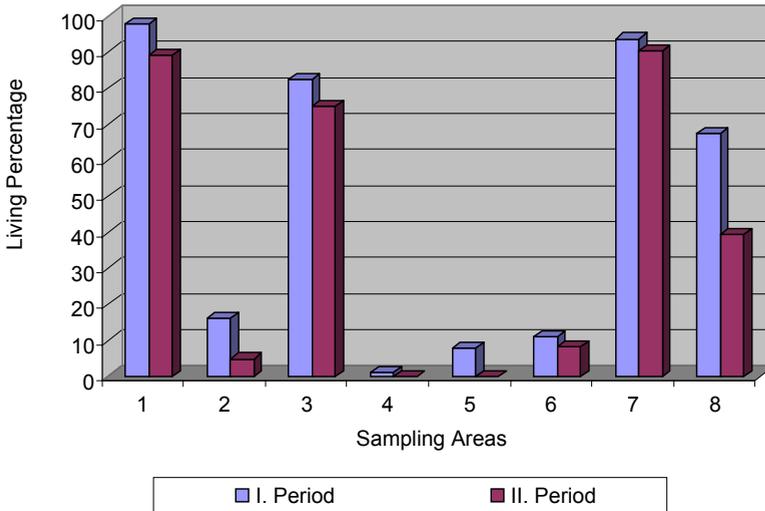


Fig. 1. Evaluated living percentages of cones in the first and the second periods in the sampling areas.

Table 2. Percentage of cones (one, two, three aged) affected by insects

Year	Cone age	Evaluated cone number	Percentage of cones (one, two, three aged) affected by				
			<i>Dioryctria pinea</i>	<i>Ernobius pini</i>	<i>Tomicus</i> sp.	<i>Camptomyia pinicola</i>	Thrips species
2005	1	749		0.4		0.26	0.26
	2	453					
	3	65	1.5				
2006	1	1401		0.07			0.14
	2	378		0.52			
	3	520	0.76			0.38	
2007	1	697		0.14			0.14
	2	586		1.19			
	3	393	2.79			0.76	
2008	1	537		0.18	0.74		0.55
	2	141		9.92			
	3	616	3.57			0.64	

Dioryctria pineae Staudinger (Lepidoptera, Pyralidae). *D. pineae* individuals were obtained from two and three aged cones in the 3rd, 5th and 8th sample areas. Especially in the third sample area 3% of

three aged cones were infested by this pest. Adults emerged from the beginning of September till the end of October.

Ernobius pini Sturm (Coleoptera, Anobiidae). This pest was found in dried one aged conelets from the previous year, drying two aged cones and also in drying or in *D. pineae* damaged three aged cones. Adults emerged during June and July. It was observed that beside matures, larvae also kept on feeding at dried material along the year.

Tomicus sp. (Coleoptera, Curculionidae). In this study, *Tomicus* sp. was found in the 1st, 2nd and 8th sampling areas with a very low population rate. Adults emerged from the beginning of March to the middle of April.

Camptomyia pinicola Mamaev (Diptera, Cecidomyiidae). This species was observed in three aged cones damaged by *D. pineae* in the 3rd and 8th sampling areas. It is accepted as a secondary pest since it was found together with other pests. Adults emerged from mid-April to mid-November.

Cicada sp. (Homoptera, Cicadidae). This pest was observed in all sampling areas with a high population rate. Adults emerged from the end of June to the end of August.

Apterygothrips priesneri zur Strassen (Thysanoptera, Phlaeothripidae) and *Neohydatothrips gracilicornis* (Williams) (Thysanoptera, Thripidae). These species were found in the 3rd and 5th sample areas. The number of insects observed was very low, specially for *N. gracilicornis*.

Sordaria sp., *Pestalotiopsis* sp. and *Dothistroma* sp. were recorded as the fungal diseases especially in the 8th sampling area.

IV – Discussion and conclusions

To investigate the relation between the cone losses and pest-disease effects, 8 different sample areas in Kozak catchment area were examined during 2005-2008.

As the result, some needle and cone pests and diseases were identified. But neither a pest nor a disease was found as the effect of the high losses.

The 2nd, 4th, 5th and 6th sample areas had lower living percentages of cones than the other areas (Fig. 1). The needle lengths in those sampling areas were shorter and sparse. This was probably related with the lack of water in the summer months and the microclimatic conditions. And there was no evidence between one aged cone losses and insect pests. This situation in one-year aged cones, was explained by Roques (1977) with progeny incontinence (=conelet abortion) because of different abiotic factors. Katowich *et al.* (1989) indicated that 0-4 month interval was the major period of losses in the seed orchard, and conelet abortion as the major mortality factor. The results of the present work show the same.

By the way, in the 1st, 2nd and 8th sampling areas *Tomicus* sp. was responsible for conelet losses indirectly but in lower population level. This pest should be kept under observations in the 8th sampling area, since it tends to be more active together with the stress factors such as drought.

On the other hand, the pests and damage rates obtained from two- and three-year aged cones are parallel with the former studies.

The most abundant insect species on two and three aged cones was *Dioryctria pineae* Staudinger (Lep., Pyralidae). This pest was found in the 3rd, 5th and 8th sample areas and its damage rate reached to 3% of three-year aged cones in the 3rd sample area. The results of former studies on the cone pests in stone pine and other pine species (Roques, 1983; Dormont and Roques, 1999; Kanat, 2001; Can ve Özçankaya, 2006; Mutke *et al.*, 2007) determine *Dioryctria* species as the key pests. According to Mutke *et al.* (2007) damaged cones by *D. mendacella* can be easily sorted out by their changed colour, burned and brownish. But, some natural enemies of this pest like *Carpelimus* sp. (Col., Staphylinidae) and some parasitic hymenopter species were observed

abundantly during this study. This datum indicates that the natural balance between the pest and the natural enemy populations is conserved. The damage of *D. pineae* remains at acceptable rates when the natural enemy population is considered.

Tomiscus sp. (Col., Curculionidae) caused indirect conelet losses –making some damages on shoots– with low population level. However, it should be kept under observation, because it tends to be more active together with some stress factors such as drought.

Other pests were identified, as *Ernobius pini* (Col., Anobiidae) and *Camptomyia pinicola* (Dipt., Cecidomyiidae) at the different aged cones in all sampling areas. These pests were defined as secondary pests since they were found in damaged cones by *D. pinea* or abiotic factors.

Cicada sp. (Hom., Cicadidae) was found abundantly in all sampling areas. But the relationship between cone losses and this pest should be examined with more details because stone pine trees are bigger than the other host plants of *Cicada* species, consequently showing a higher tolerance to pests.

In addition, *Sordaria* sp., *Pestalotiopsis* sp. and *Dothistroma* sp. were recorded as the fungal diseases. *Dothistroma* needle blight of pine was determined in some areas in some years. Sinclair *et al.* (1989) reported that the disease had made a severe damage in seedlings and young plantations of *Pinus radiata*, *P. nigra* and *P. ponderosa* in New Zealand and North America. Besides, *Sordaria* sp. and *Pestalotiopsis* sp. which are common fungal diseases were identified in the 8th sample area. Even, the symptoms of *Pestalotiopsis* sp. have been observed in needles and shoots weakened or injured by other factors such as freezing, sun burn or insects, and as the result, they wilt and turn brown (Sinclair *et al.*, 1989).

When summarized, some pests and diseases of needle and cones have been determined in the whole sampling areas. But none of them could cause significant cone loss. However, it should be taken into consideration that pests and diseases could become more effective in adverse climatic conditions such as drought and increase in the number of stone quarries in the province.

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Cone yield evaluation of a grafted *Pinus pinea* L. trial

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Abstract. Grafted stone pine plantations for cone production could be an interesting alternative on low quality agriculture lands, as they display various advantages compared to the traditional forest harvesting: the possibility to use more productive genotypes, early bearing, easily harvesting, etc. Some grafted experiences with *P. pinea* have already been done, mainly focused on elucidating the relevance of environmental factors and in the selection of the best productive genotypes; however the productive information is still scanty. With a view to go into the species' agronomical potential knowledge in depth, in 2008 a grafted trial was planted at IRTA's Torre Marimon station (Caldes de Montbui, Spain). In 2010 an irrigation essay was started in order to study the effect of different water regimes on the strobili induction and productive responses. The first results show an early production onset on production, from barely 50 flowers/ha in 2008 to more than 3,500 in 2011. The first significant cone yield was registered in 2011, with 840 kg·ha⁻¹. Concerning water supply, preliminary results show in the irrigated trees a lower fruit mortality on the second development year than in the control (7% against 20%, in 2010) and a higher floral induction (14 flowers/tree against 10, in 2011). Further results in the coming years will allow to evaluate the productive potential of this kind of plantations.

Keywords. Stone pine – Pine nut – Orchard management – Irrigation.

Evaluation productive d'une plantation greffée de *Pinus pinea* L.

Résumé. Les plantations greffées de *Pinus pinea* peuvent constituer une intéressante alternative pour les terrains agricoles de basse qualité car présentant divers avantages en comparaison à la gestion forestière traditionnelle : possibilité d'utiliser des génotypes plus productifs, rapide entrée en production, facilité de récolte, etc. Diverses expériences de *P. pinea* greffé ont été déjà faites, principalement orientées à déterminer l'importance des facteurs environnementaux et à sélectionner des génotypes plus productifs. Cependant, l'information productive est plutôt rare. Afin d'approfondir les connaissances sur le potentiel agronomique de l'espèce, une plantation de *P. pinea* greffé fut établie en 2008 à l'IRTA Torre Marimon (Caldes de Montbui, Espagne). En 2010 un essai d'irrigation démarra dans l'objectif d'étudier l'effet de différents régimes hydriques pour l'induction florale et la réponse productive. Les premiers résultats montrent une rapide entrée en production, passant de 50 fleurs/ha en 2008 jusqu'à plus de 3500 en 2011. La première production significative de cônes eut lieu en 2011, avec 840 kg/ha. Concernant l'apport hydrique, les résultats préliminaires montrent une plus faible mortalité des cônes de deuxième année (7% versus 20%, en 2010) et une plus nombreuse induction florale (14 fleurs/arbre versus 10, en 2011) des arbres irrigués par rapport aux arbres témoins. Le suivi de cette expérience permettra d'évaluer plus précisément le potentiel productif de ce type de plantations.

Mots-clés. Pin pignon – Pignon – Gestion des vergers – Irrigation.

I – Introduction

In spite of the high commercial value of its edible kernels, the Mediterranean stone pine still remains a genuine forest species. At present, the virtually total commercial cone yield is still harvested from natural or naturalised forests (Mutke *et al.*, 2000). Grafted plantations for cone production could be an interesting alternative on low quality agriculture lands. These plantations display various advantages compared to the traditional forest harvesting: early bearing, the possibility to use more productive genotypes and more adapted rootstocks to the soil, easily harvesting, better control against cone pillage, etc.

Some grafted plantations and trials with stone pine have already been done; however their productive information is still scarce. The oldest correspond to reforestations transformed by grafting. However, their cone yield evaluation was discontinuous and consequently provided imprecise data. One of the first documented experiences in Spain took place in the eighties (Catalan, 1990). More recently, experimental clonal orchards have been established since early nineties (Mutke *et al.*, 2000), mainly focused on elucidating the relevance of environmental and genetic factors for seed-yield quantity and quality (Mutke *et al.*, 2003; Mutke *et al.*, 2005a; Mutke *et al.*, 2007). With a view to go further in the study on the species' agronomical potential, a grafted *P. pinea* trial was planted in 2008 at IRTA's Research Station of Torre Marimon, near Barcelona.

Stone pine has a strong masting habit, which entails a very irregular fruitfulness in natural stands (Mutke *et al.*, 2005b). Thus, a particular objective of this trial is to assess the effect of watering in floral induction, cone survival throughout the three-year development cycle, and in seed-yield quantity and quality. In other words, how alternate bearing is affected by watering.

II – Material and methods

1. Site description and plant material

The trial plot is located at IRTA's station Torre Marimon (Caldes de Montbui, Barcelona), at 2° 10' E, 41° 37' N and 160 m a.s.l. on the Pre-littoral Depression. The soil has a sandy texture with a clay horizon in the middle of the profile, low fertility and a lightly alkaline pH. Average annual temperature is 14.9°C, with absolutes from –8.6 to 39.7 °C (2000-2010). The average annual rainfall is 624 mm (1991-2000) and ranges from 472 to 954 mm. The climate is Nemoral oromediterranean in transition with Subnemoral Mediterranean, according to the Phytoclimatic Atlas of Spain (Allué Andrade, 1990).

The trial was established in spring 2008; 96 grafts were planted in a 6 x 6 m setting, thus the plot occupies 0.35 ha. Plants had been produced in 2003 by cleft grafting on seed-grown rootstocks of *P. halepensis* (heteroplastic grafts) stone pine scions collected from a group of 10 trees (from Catalonia Littoral provenance region) and remained in nursery for five years, stocked in very limiting conditions. Prior to the plantation, a soil preparation was carried out, including manuring and deep-plowing with a ripper. Drip irrigation was installed and weed competence was controlled.

2. Irrigation experiment

For the design of the irrigation trial, the results of previous studies have been taken into account. Water stress seems to be the most notable limiting factor in stone pine cone-yield (Mutke *et al.*, 2005b) and there is a correlation of rainfall at the end of shoot elongation (June rainfall) with shoot length and flower bearing in the next year (Mutke *et al.*, 2003). According to these statements, the growing season has been divided in 3 periods in spring, early summer and late summer (Table 1), considering physiological processes involved, and irrigation treatments have been established considering different application periods: irrigation treatment 1 (T1) covers periods 1 and 2, and irrigation treatment 2 (T2) only covers the spring period 1 (Table 2). Outer rows of the trial constitute a control treatment, only watered in extreme drought events. Water supply (difference between rainfall and crop evapotranspiration of the previous week) is calculated according to rainfall and reference evapotranspiration (ET_o) data registered by the Torre Marimon weather station. Crop evapotranspiration (ET_c) is defined as the combined water vapour leaving the system by evaporation from soil and plant surfaces, which is calculated by multiplying ET_o by a crop coefficient, K_c (White and Fisher 1985; Allen *et al.*, 1998).

Table 1. Periods of the growing season and physiological processes that will be affected by irrigation treatments

Period	Dates *	Processes involved	
Period 1	April 1 – May 31	Spring shoot elongation 2nd year cone growth 3rd year cone growth	Secondary growth Flowering and pollination
Period 2	June 1 – July 31	Terminal buds differentiation Fertilisation Occasional summer shoot growth	Needle growth Secondary growth (final) 3rd year cone growth (final)
Period 3	August 1 – September 31	Needle growth (final) Occasional summer shoot growth	Embryo development

*Dates are approximate and must be corrected according to the year's phenology.

Table 2. Definition of the irrigation treatments

Irrigation treatment	Period 1	Period 2	Period 3
T1	100% [P-ETc]	100% [P-ETc]	*
T2	100% [P-ETc]	*	*
Control	*	*	*

*Only watered in extreme drought events. P: rainfall; ETc: crop evapotranspiration.

The trial design was on randomized complete blocks, with 3 repetitions and 2 trees per observation. The plantation began to be watered in 2009, and the water supply was 64 litres·tree⁻¹·week⁻¹, from April until August. In 2010 the irrigation system in lateral rows (control) was stopped; the rest of the trail was irrigated homogeneously with 64 litres·tree⁻¹·week⁻¹ from June to August. In 2011 the irrigation treatments have been properly applied in the trail for the first time: 115.6 m³·ha⁻¹ of water was supplied on the T1 and 55.6 m³·ha⁻¹ on the T2 (Fig. 1). These quantities belong to a young plantation with 5% of canopy cover.

3. Measurements

Since plantation, several variables are measured at individual tree level: stem diameter above the graft union (used as covariate to adjust individual cone yield); crown projection (used to know the plant cover, variable needed in irrigation calculations); phenology of shoot and flower development, used to adjust beginning and end dates of the irrigation periods and also to detect the presence of summer shoots and second female flowering (polycyclic growth); cone cohorts have been monitored from initial female strobili number until the total number of ripe cones. Size, weight and number of seeds of each individual cone, seed yield (kg) and seed output are measured only in monitored trees (two trees per treatment and replication, i.e. a total of 18 trees).

III – Results and discussion

1. Onset on production

The first results of the trial show an early onset on production of trees, from barely 50 strobili/ha in 2008 to more than 3,500 produced in 2011 (Fig. 2). The first significant female flowering (with around 1,900 strobili/ha) took place in 2009, and its corresponding cone yield, in 2011 (4 years

after planting). First male flowering appeared in 2011 (4 years after planting), but in low intensity. However, lateral pollination from surrounding adult trees guaranteed cone setting since the first flowering.

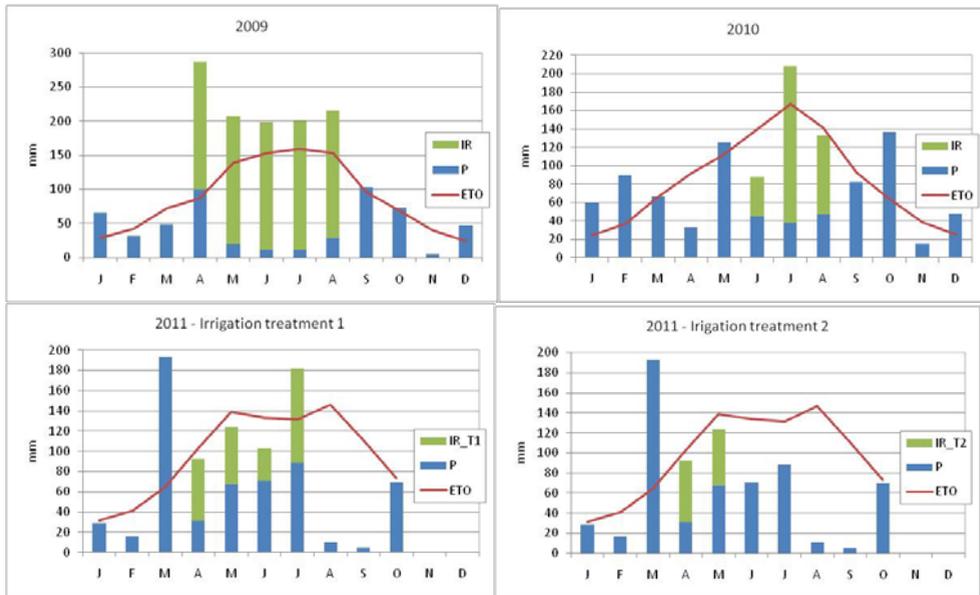


Fig. 1. Distribution of rainfall, evapotranspiration and water supplied since the beginning of the experience (IR: irrigation; P: rainfall; ETO: reference evapotranspiration).

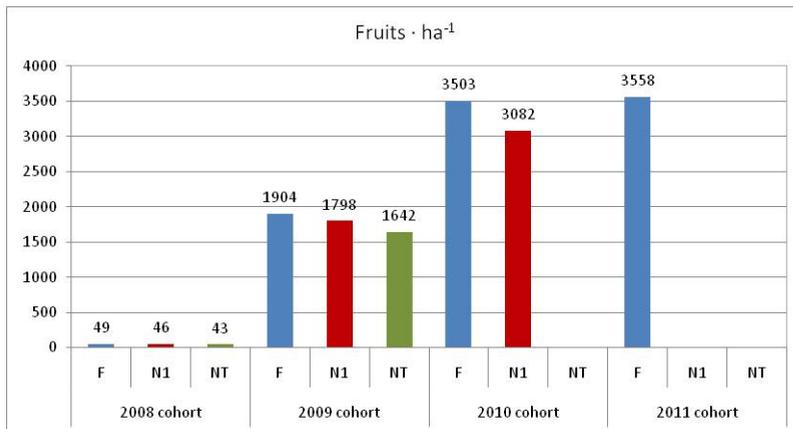


Fig. 2. Evolution of the successive reproductive cohorts (F: number of pollinated conelets; N1: number of one-year old cones; NT: total number of ripe cones, corresponding to the fruit evolution of three consecutive years).

The consecutive yield variables along the pathway from initial number of female buds up to the cone and seed yield are shown in Table 3. Concerning the first representative cone yield (2009 cohort), cone survival throughout the tree years (NC/IF) was about 76%; 290 kg of sound cones

were harvested in the trail, which would be equivalent to 839 kg·ha⁻¹; the mean tree yield (PC/96) was 3.0 kg·tree⁻¹ and the mean cone weight (PC/NC) 0.58 kg·cone⁻¹.

Table 3. Successive cone and seed yield parameters of the reproductive cohorts (total plot size 96 trees)

Cohort	IF	F	N0	N1	NT	NC	PC	PP
2008	-	17	17	16	15	15	3.7	0.8
2009	660	647	637	623	569	504	290.1	
2010	1214	1192	1068	1051				
2011	1240	1233	1038					

IF – initial female conelets number; F: number of pollinated conelets; N0: number of cones surviving the first summer; N1: number of one-year old cones; NT: total number of ripe cones; NC: number of sound cones; PC: cone yield (kg); PP: seed yield (kg).

2. Effects of watering

Concerning the effects of water supply only preliminary results have been arisen. Results of 2011 cone yield (Table 4) show a significant irrigation effect on the mean individual cone yield (4 kg·tree⁻¹ in T1 against 1.9 in control), and also on the mean cone weight (616 g·cone⁻¹ in T1 against 536 in control). However, according to the 2011 harvest data, the prolongation of the water supply until half-end of July does not show a significant cone yield increment, nor an individual cone weight increase.

Table 4. Analysis of variance for 2011 mean cone yield and mean cone weight

Irrigation treatment	Mean cone yield (kg·tree ⁻¹)	Mean cone weight (kg·cone ⁻¹)
T1	4.0a	0.616a
T2	3.4a	0.592a
Control	1.9b	0.536b
Significance	***	*

Duncan ($\alpha=0.05$); *: significant at $P<0.05\%$; ***: significant $P<0.001\%$. Same letter indicates no significant differences between treatments.

Extrapolating the mean tree yield into yield per hectare, we would have obtained 509, 1056, 833 kg·ha⁻¹ in treatment control, treatment 1 and treatment 2 respectively (Table 5). Even in control treatment, the cone yield would be higher than 500 kg·ha⁻¹. The average production in Spanish natural adult stands ranges from 200-600 kg·ha⁻¹ year. The average yield from data recorded in public forests of Valladolid province (Northern Inland Plateau) from 1960 to 2000 is 193 kg·ha⁻¹ (Mutke *et al.*, 2005b). The higher yield achieved in this trial is not only due to the irrigation effect; other factors are involved: grafted plants, site preparation, canopy illumination, herbaceous competition control, mild climate, etc. But, in 2011, a higher floral induction in irrigated trees against those without water supply was significantly detected (14 flowers·tree⁻¹ against 10 flowers·tree⁻¹; $P<0.001$).

Table 5. Yield variables for the different irrigation treatments

Yield variable	Control	T 1	T 2
Mean tree yield (kg-tree ⁻¹)	1.9	4.0	3.4
Sound cone yield (kg·ha ⁻¹)	509	1,056	833
Estimated seed yield (kg·ha ⁻¹) [†] (20% of cone weight)	101.7	211.2	166.5
Estimated unshelled seed yield (kg·ha ⁻¹) [†] (5% of cone weight)	25.4	52.8	41.6

[†]The evaluation of cone and seed variables of the first cone yield is currently in progress.

Table 6. Analysis of variance for 2011 average individual flower number

Treatment	Average individual flowers number (flowers·tree ⁻¹)
Irrigated	13.92a
Control (non-irrigated)	9.96b
Significance	***

Duncan ($\alpha=0.05$); ***: significant at $P<0.001\%$. Same letter indicates no significant differences between treatments.

3. Water requirements estimation

Average monthly data of precipitation and ETo from the Torre Marimon weather station (period 1991-2010) are shown in Fig. 3. From these data, ETc, water deficit and water supply have been calculated (Table 7). These data show that months with water deficit correspond to the 3 periods in which the growing season has been divided. Thus, it is estimated that average water requirements for compensating the water deficit in our environmental conditions are 136 m³·ha⁻¹·year⁻¹ for treatment 1 and 35 m³·ha⁻¹·year⁻¹ for treatment 2. These values refer to a 5% canopy cover. For a 50% of canopy cover, the theoretical average water supply would be around 1,300-1,400 m³·ha⁻¹·year⁻¹. It is considered that more than 50% canopy covers are not recommended for this kind of plantations, in order to maintain a proper canopy illumination and to guarantee floral induction. Anyway, these are low amounts of water supply compared with other nut crops, like almond tree, which annual water demand is calculated around 2,000 m³·ha⁻¹·year⁻¹ in a regulated deficit irrigation scheduling (Girona *et al.*, 2005).

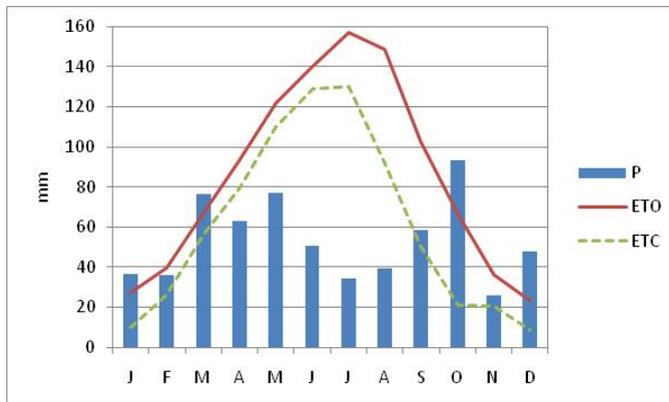


Fig. 3. Average distribution (1991-2010) of precipitation and evapotranspiration in the trial plot (P: rainfall; ETo: potential evapotranspiration; ETC: crop evapotranspiration).

Table 7. Calculation and distribution of water requirements

	P (mm)	ET _o (mm)	K _c	ET _c (mm)	P-ET _c (mm)	WS (m ³ ·ha ⁻¹)	Irrigation period	Irrigation treatment
Jan	36.3	27.5	0.35	9.6	26.7			
Feb	35.7	39.5	0.66	26.1	9.7			
Mar	76.3	67.3	0.84	56.5	19.8			
Apr	63.2	93.4	0.85	79.4	-16.1	12.5	Period 1	T1, T2
May	76.8	121.9	0.9	109.7	-32.9	22.5	Period 1	T1, T2
Jun	50.6	140.0	0.92	128.8	-78.5	46.1	Period 2	T1
Jul	34.1	157.0	0.83	130.3	-96.2	55.2	Period 2	T1
Aug	39.3	148.5	0.62	92.1	-52.8	31.4	Period 3	
Sep	58.7	102.7	0.49	50.3	8.4		Period 3	
Oct	93.5	66.9	0.32	21.4	72.1			
Nov	25.7	36.6	0.56	20.5	5.2			
Dec	47.9	23.5	0.38	8.9	39.0			

P: rainfall; ET_o: reference evapotranspiration; K_c: crop coefficient; ET_c: crop evapotranspiration; WS: water supply

The productive potential of this kind of plantations is expected to be evaluated by further results, establishing a guide for maximizing production and also for cone quality improvement.

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Production and management of stone pine (*Pinus pinea*) for early nut production: grafted plantations as an alternative for restoring degraded areas and generating income in rural communities of Tunisia

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Abstract. We introduce a four years project (2008-2012), supported by the Program of Scientific Cooperation and Interuniversity Research (PCI), funded by the Spanish Agency for International Development Cooperation (AECID), and implemented in Tunisia and Spain. The objective of the project was to establish a systematic methodology for producing grafted plant using two types of stock: *Pinus pinea* and *Pinus halepensis*, from known provenances from Spain and Tunisia and to implement field trials in both countries, on different hydric and edaphic conditions, for evaluating the development of the plantations. Due to *Pinus pinea* interest for nut production, grafted *Pinus pinea* plantations for early and abundant cone yield can be an interesting alternative for restoring and revalorize some Mediterranean areas, generating both economic and ecological benefits (soil protection).

Keywords. Cooperation project – *Pinus halepensis* root-stocks – Nursery – Spain.

Production et gestion du pin pignon (*Pinus pinea* L.) pour une production précoce de pignons : les plantations greffées comme alternative pour la restauration de zones dégradées et pour la création de revenus chez des communautés rurales de Tunisie

Résumé. Nous présentons ici un projet quadriennal (2008-2012), mené avec l'appui du Programme de Coopération Interuniversitaire et de Recherche Scientifique (PCI), financé par l'Agence Espagnole pour la Coopération Internationale au Développement (AECID), et mis en place en Tunisie et en Espagne. L'objectif de ce projet était d'établir une méthodologie systématique pour produire des plants greffés en utilisant deux types de porte-greffe : *Pinus pinea* et *Pinus halepensis*, de provenances connues d'Espagne et de Tunisie, et de mettre en place des essais de terrain dans ces deux pays, sous différentes conditions hydriques et édaphiques, afin d'évaluer le développement des plantations. Dû à l'intérêt de *Pinus pinea* pour la production de pignons, les plantations greffées de *Pinus pinea* pour une production précoce et abondante de cônes peuvent constituer une alternative intéressante afin de restaurer et de revaloriser certaines zones méditerranéennes, créant ainsi des bénéfices autant économiques qu'écologiques (protection du sol).

Mots-clés. Projet de coopération – Porte-greffe *Pinus halepensis* – Pépinière – Espagne.

I – Introduction

Pinus pinea is one of the most appreciated species in the Mediterranean basin due to the multiple products and functions it offers (timber and fruit production, soil protection, biodiversity, or landscape). Between them the most interesting from the economic point of view is the pine

nut production. In Spain and Tunisia there is a great interest from forest managers in how to improve *Pinus pinea* cones production.

In this sense, the grafted plantations can be a good chance for restoring degraded areas and generating income in rural communities in both countries. Moreover, grafting *Pinus pinea* on *Pinus halepensis* rootstocks can result interesting for their use in semiarid calcareous sites where their root system is supposed to be better adapted – one of the objectives of the presented project was the establishment of field trials about this question.

In Tunis, *Pinus pinea* has been planted since 1907 in reforestation programs, being the most important specie after *Pinus halepensis*, occupying about 20,000 ha. To the present, the potential of grafted plantations for cone production has not been evaluated, though its interest motivated the preparation of a project, for resolving questions about grafting techniques and plantation management.

The main objectives of the project were:

- (i) to strengthen institutionally INRGREF in order to promote its research and training capacities with regard to production and management of grafted stone pine,
- (ii) to establish a systematic methodology for producing grafted plant using two types of stock: *Pinus pinea* and *Pinus halepensis*, from known provenances from Spain and Tunisia,
- (iii) to assess grafting success on both rootstock types and grafting techniques,
- (iv) to evaluate the adaptation of different rootstocks to different water and soil conditions at nursery,
- (v) to produce grafted stone pines from selected Spanish and Tunisian vegetative materials for the establishment of new plantations,
- (vi) to implement field trials in both countries, under different water and soil conditions, evaluating the development of the plantations,
- (vii) to share and transfer techniques and results.

II – Stages of the project

During the first year of the project (2008) the research teams from both countries came together to update available knowledge, revise the methodology and reinforce capacities for vegetative materials collection. In Spain, a first grafting trial in nursery was established (Fig. 1).



Fig. 1. Left: *Pinus halepensis* and *Pinus pinea* stocks prepared for grafting. Right: Scion selection for grafting in Spanish clone banks.

In 2009, we focused on the selection and characterization of vegetative materials and stock production under controlled nursery conditions. Experimental grafting experiences in Spain made possible to transfer this technique during a visit of the Spanish team to Tunisia (Fig. 2). A field trial under controlled conditions with Spanish grafted material was also done.



Fig. 2. Left: *Pinus halepensis* and *Pinus pinea* stock production in Tunisia. Right: Grafting in Tunisia using selected Spanish scions.

In 2010, the trials implemented in Spain during the previous years were followed and further selection of vegetative materials and new nursery experiences were performed (Fig. 3).



Fig. 3. Left: Scion collection for grafting. Right: Stocks production for nursery experiences (water stress trial) in Spain.

Also in 2010, in Tunisia a greenhouse was built and equipped, in order to implement further trials and to produce suitable grafted materials. Despite a low grafting success, the systematic methodology was consolidated and the Tunisian team could start training local staff (Fig. 4). Another visit to Tunis for grafting transfer was done.



Fig. 4. Left: Grafting technique transfer in Tunisia. Right: Stocks production for nursery experiences (test of soil conditions) in Tunisia.

During 2011, field trials under natural conditions were implemented in Spain and Tunis (Fig. 5) and the data obtained from the experiences were processed. A planned trip of the Spanish team to Tunisia in March was postponed one year due to the political situation in Spring 2011.



Fig. 5. Grafted plantations installed in Spain (left) and Tunisia (right).

In March 2012 finally, the trip to Tunisia was done for grafting selected Spanish and Tunisian material for the establishment of new plantations at the end of the year, continuing the instruction of Tunisian grafters (Fig. 6). Also, during the visit we worked in the preparation of a final conference, for sharing experiences and results emerging from the project, which may enhance research and transfer capacities.



Fig. 6. Grafted material in Tunisia (2012).

In the project several coordination meetings, techniques transfer and information exchange have been done in both countries (Fig. 7).



Fig. 7. Left: Meeting in Solsona, November 2008. Right: Final conference in Tunis, March 2012.

In the final meeting conference, held in June 2012 at the National Gene Bank in Tunis, main results of the project were presented, in relation to:

(i) The adaptation of the different provenances of *Pinus pinea* and *Pinus halepensis* stocks, from Tunisia and Spain, to different water stress conditions.

(ii) Success of *Pinus pinea* grafting on *Pinus pinea* and *Pinus halepensis* root-stocks from Tunisia and Spain.

(iii) Experiences of *Pinus pinea* grafted plantations installed in Tunisia and Spain (planted material: *Pinus pinea* grafted on *Pinus pinea* and *Pinus halepensis* root-stocks from Tunisia and Spain).

Part of these results have been presented also at the AGROPINE2011 Meeting on Mediterranean stone pine for Agroforestry held in Valladolid, and in the present issue of Options Méditerranéennes.

III – Conclusions

The collaboration between both countries has resulted very interesting and beneficial. Despite some logistic problems and delays in some phases of execution, as the building and equipment of a nursery in Tunisia or the field plantations, the main objectives of the project has been obtained successfully.

We hope that the result of this technical and scientific collaboration will promote research and training capacities with regard to production and management of grafted stone pine, in order to promote its plantations as an alternative for restoring degraded areas and generating income in rural communities of Mediterranean basin, especially in Tunisia.

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Thinning effect in two young stone pine plantations (*Pinus pinea* L.) in central southern Chile

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Abstract. In Chile stone pine was introduced more than a century ago, mainly by Italian and Spanish immigrants who brought it as part of their culture; interest for this nut in Chile is recent and has been gradually increasing. Crown development is one of the most important factors on pine nut production because it is influenced mainly by light. In winter 2009 two young *P. pinea* plantations, 15 and 16 years old, located in central Chile (Casablanca –Valparaíso Region– and Pehuenhue –Maule region), were thinned in order to evaluate the effect of this intervention. Variables evaluated were DBH, height and crown diameter. With the software ArcGIS 9.2, an analysis of crown area growth was made before and after thinning. Results showed a positive increase in dasometric variables one and two years after the intervention in both situations. A significant increase in tree crown area was found; crowns tend strategically to take advantage of light and space, taking a rounded form, change that would have a significant effect on pine nut production.

Keywords. Crown area – *Pinus pinea* – Pine nut production – Stone pine – Thinning.

Effet de l'éclaircie dans deux jeunes plantations de pin pignon (*Pinus pinea* L.) dans le centre sud du Chili

Résumé. Au Chili le pin pignon a été introduit il y a plus d'un siècle, principalement par des immigrants italiens et espagnols qui l'ont amené dans le cadre de leur culture, mais au Chili l'intérêt pour cette noix est récent et progressif. Le développement du houppier est l'un des facteurs ayant le plus d'impact sur la production de pignons, car celle-ci est principalement influencée par la lumière. Pendant l'hiver 2009, deux jeunes plantations de *P. pinea*, de 15 et 16 ans, situées dans le centre du Chili (Casablanca, région de Valparaíso, et Pehuenhue, région de Maule), ont été éclaircies afin d'évaluer l'effet de cette intervention. Les variables évaluées sont la hauteur, le diamètre et la projection de la surface du houppier. Plus tard, avec le logiciel ArcGIS 9.2, une analyse de la croissance de la surface du houppier projetée avant et après éclaircie a été réalisée. Le résultat fut une augmentation positive des variables dasométriques un et deux ans après l'intervention dans les deux situations. On a trouvé une augmentation significative de la surface du houppier des arbres, qui tendent de manière stratégique à profiter de la lumière et de l'espace, formant un houppier arrondi, changement qui aurait un effet significatif sur la production de pignons.

Mots-clés. Eclaircie – *Pinus pinea* – Production de pignons – Surface du houppier.

I – Introduction

The forestry sector in Chile has been focused primarily on production of mainly two species, *Pinus radiata* and *Eucalyptus* spp. However, currently there is a great interest of public and private stakeholders in the diversification of productive species, not only for producing timber and cellulose, but also to identify sustainable alternatives, especially for medium and small landowners.

An alternative to traditional forest activity is promoting the production of non-timber forest products (NTFPs), among which pine nuts from stone pine (*Pinus pinea* L.) are an interesting option. This species is a conifer with edible seeds called "pine nuts", highly demanded, native to

the Mediterranean area; it requires between 400-800 mm of annual rainfall with winter rainfall regime and four to six dry months (Mutke *et al.*, 2007).

Stone pine is characterized by an abundant and rounded crown; fruit bearing begins at 15-18 years in its origin area. Female flowers are placed on vigorous shoots of the year in the upper crown area, so many authors describe that larger trees have greater vigor and flowering (Gordo *et al.* 1999; Loewe *et al.*, 2011).

In Chile, stone pine was introduced more than a century ago, mainly by Italian and Spanish immigrants who brought it as part of their culture, being used primarily to fix dunes, control erosion and provide shade for livestock, but the interest for its fruit is recent. The Chilean Forestry Institute (INFOR) has studied the species since 1994 and more recently, since 2008, is conducting the project "The edible pine nut of stone pine (*Pinus pinea* L.), an attractive business for Chile", in order to design, implement and evaluate productive models to cultivate stone pine oriented to pine nut production, or combined to produce wood and fruit, and to analyze productivity, nut quality, among other topics.

As part of these activities, management trials have been carried out on pre-existing plantations, considering pruning and thinning, with interesting results (Loewe *et al.*, 2011). The objectives of thinning *P. pinea*, as Montero and Candela (1998) state, correspond to control mass density in different stages of development in order to maximize production and profits, improving plant vigor and quality of the remaining trees, and to obtain secondary products that have a market value.

In traditional timber producing species the main objective of thinning is to increase tree diameter and height growth, in order to obtain higher yields and better quality timber (Sotomayor *et al.*, 2002). Nevertheless, in species whose goal is fruit production, such as *P. pinea*, thinning can be done to increase crown growth and tree biomass which will also increase fruit production. According to Muñoz *et al.* (2008), crown increase allows higher amount of light to be intercepted by the canopy, improving productivity and stand dynamics.

In this context, the goal of this study was to evaluate the thinning effect in growth parameters of the species at two locations in central Chile, in order to stimulate the production of pine nuts due to its remarkable value in the international market.

II – Material and methods

The study area corresponds to two sites located in the central region of Chile, Casablanca and Péncahue.

1. Casablanca

The plantation located in Casablanca (latitude 33°22'37"S, longitude 71°19'18"W), Valparaíso Region, corresponds to a European provenance trial established by INFOR in 1994. The plantation, 0.6 ha, was established on a site with minimal slope (0-2%), with an initial spacing of 2 x 3 m; it has three blocks of six plots each (49 trees per plot). Provenances are Lombardy and Tuscany (Italy); Slovenia; and Meseta Castellana, Western Andalucía and Sierra Morena (Spain).

In July 2009 the plantation was thinned extracting 47.8% of all alive trees (mortality was 30% at that moment). Prior to thinning, for each tree it was measured height, DBH and crown diameter on NE and SW orientations.

2. Péncahue

Plantation located in Toconey (latitude 35°24 '42"S, longitude 72°3'32"W), county of Péncahue,

located in the Maule River Valley, Maule Region, established on 1993. The plantation, 1.8 ha, was established in a northern exposure hillside with a slope of 20-25% at a spacing of 5 x 5 m.

In winter 2009, a thinning was done, extracting rows diagonally (geometric thinning), achieving a final average spacing of 7 x 7 meters.

The evaluation was performed considering three different exposure plots: P1). Northwest, 52 trees; P2). North, 53 trees; and P3). Northeast, 51 trees. Before and after thinning, for each tree there were measured four crown radius, facing north, east, south and west.

At one side of each plantation there was taken as reference one GPS point in UTM projected coordinates, WGS 84 datum zone 19 south, and with an image of Google Earth there was made a tree distribution map. In both situations, with the software ArcGIS 9.2, an analysis of tree crown area before (2009) and after thinning was done (2010 and 2011) in order to evaluate the effect of thinning on crown area. Data were analyzed with the Software Infostat 2009 version, and if there were significant differences, Duncan's multiple comparison test was used.

III – Results and discussion

1. Casablanca

Western Andalucia, Slovenia and Meseta Castellana, are the provenances that most increased crown area one year after thinning, with an average of 31.6% over the other three provenances, while Tuscany had the lowest increase (23% lower than the other five provenances). However, Lombardy had a greater increase two years after thinning, with a 131.7% crown area increase. Between 2010/11 the increase was lower in all provenances compared with the first period, being again Western Andalucia and Slovenia the ones that showed a better answer to thinning, with an average increase of 29.3% over the other four provenances. Two years after thinning the tendency of the period 2009/10 was the same, being Western Andalucia, Slovenia and Meseta Castellana, the ones who showed the highest crown area increase (Table 1).

Table 1. Crown area average increase in Casablanca

Provenance	Growth (m ²)					
	2009-2010	%	2010-2011	%	2009-2011	%
Meseta Castellana	2.57 ± 0.26 c	103.2	1.55 ± 0.19 bc	29.9	4.12 ± 0.34 c	159.5
Slovenia	2.32 ± 0.25 c	95.2	1.6 ± 0.23 c	32.3	3.92 ± 0.41 c	154.6
Western Andalucia	2.34 ± 0.25 c	90.2	1.49 ± 0.26 c	30.4	3.84 ± 0.35 c	144.7
Sierra Morena	2.24 ± 0.2 bc	105.1	1.09 ± 0.17 b	26.3	3.34 ± 0.23 b	159.0
Tuscany	1.70 ± 0.17 a	84.2	1.11 ± 0.16 bc	35.9	2.81 ± 0.2 ab	145.4
Lombardy	1.55 ± 0.21 ab	131.7	0.62 ± 0.13 a	25.5	2.17 ± 0.22 a	175.3

Different letters indicate significant differences between treatments according to Duncan's test ($p \leq 0.05$)

Figure 1 illustrates the thinning effect in the three blocks of Casablanca trial, highlighting the significant increase one year after thinning (2009/10). Block 3 is the one with largest increases because trees are larger due to better soil quality.

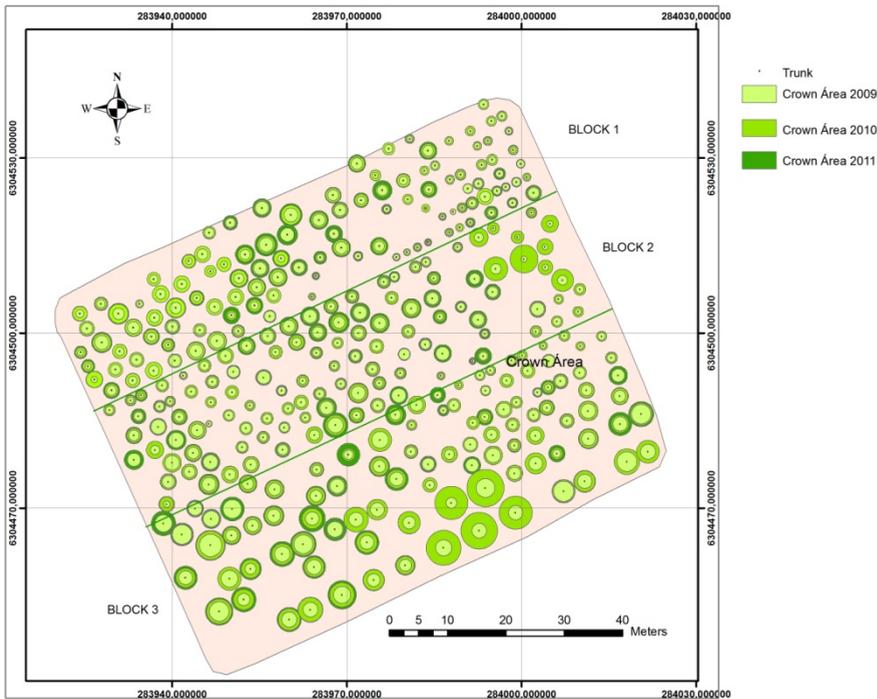


Fig. 1. Thinning effect in Casablanca stone pine trial.

DBH mean values two years after thinning show significant differences among provenances. Western Andalucía showed the highest growth two years after thinning, achieving a DBH increase of more than 3.5 cm. However, even though there are significant differences, DBH increases were similar throughout the plantation, as Meseta Castellana (provenance that showed the lower answer), increased an average of 3 cm in DBH in the same period (Fig. 2).

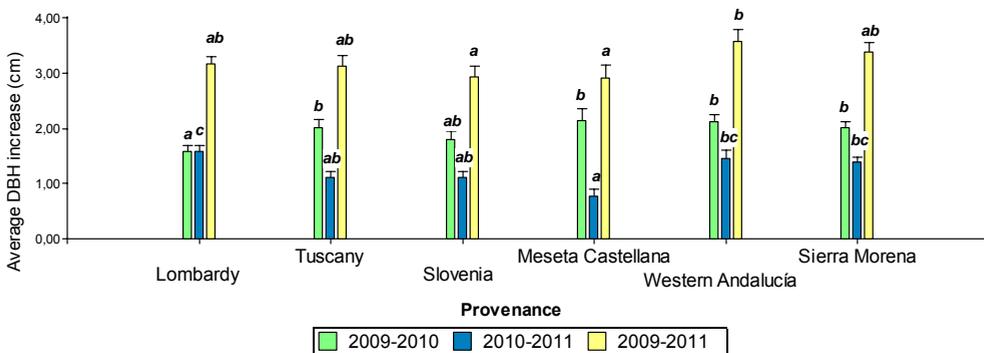


Fig. 2. DBH average increase in Casablanca trial by provenance. Different letters indicate significant differences between treatments according to Duncan's test ($p \leq 0.05$).

The results obtained two years after thinning showed statistically significant differences for

height; three provenances (Sierra Morena, Western Andalucía Meseta Castellana) were superior showing significant differences, with a 35% increase (Fig. 3).

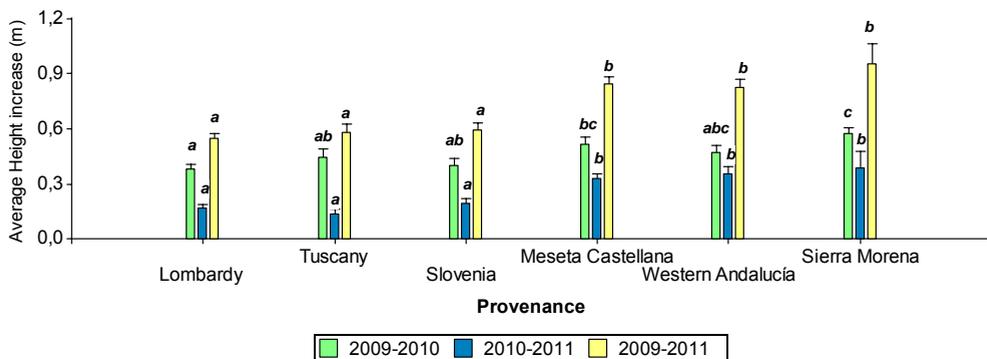


Fig. 3. Height average growth in Casablanca by provenance. Different letters indicate significant differences between treatments according to Duncan's test ($p \leq 0.05$).

2. Pencahue

Mean crown increase values showed significant differences between orientations (Table 2). The Northeast plot displayed a greater effect of thinning the second year with significant differences compared to the other plots, reaching a 93% increase. In the period 2010/11 it was observed a slight increase in crown area in all plots, ranging among 2.6 and 4 m². Again the Northeast plot had the highest significant increase (3.97 m²), and the Northwest plot achieved the highest crown area increase (15.6%).

Table 2. Crown area average increase in Pencahue

Plot	Growth (m ²)					
	2009-2010	%	2010-2011	%	2009-2011	%
Northwest	7.08 ± 0.88 a	39.1	3.73 ± 0.43 ab	15.6	10.81 ± 1.01 a	60.6
North	11.33 ± 0.87 b	65.4	2.62 ± 0.43 a	9.7	14.44 ± 1.00 b	81.2
Northeast	13.96 ± 0.90 b	72.2	3.97 ± 0.44 b	12.4	17.93 ± 1.04 c	93.0

Different letters indicate significant differences between treatments according to Duncan's test ($p \leq 0.05$).

Figure 4 illustrates the effect of thinning in the three orientations in Pencahue plantation, highlighting the significant increase the first year after thinning (period 2009/10). Plot 3 (Northeast) had the largest increases.

Results obtained two years after thinning indicate significant differences for DBH between plots; the Northwest plot presented a significant increase, 23.1% above other plots, while the Northern plot had the lowest increase for this variable (10% lower than other plots) (Fig. 5).

Mean height values two years after the intervention showed significant differences between plots/exposure. Unlike the result for DBH, the northeast plot showed the highest difference, statistically significant over the other plots in all periods, with an increase of 40% over the others in the period 2009/11 (Fig. 6).

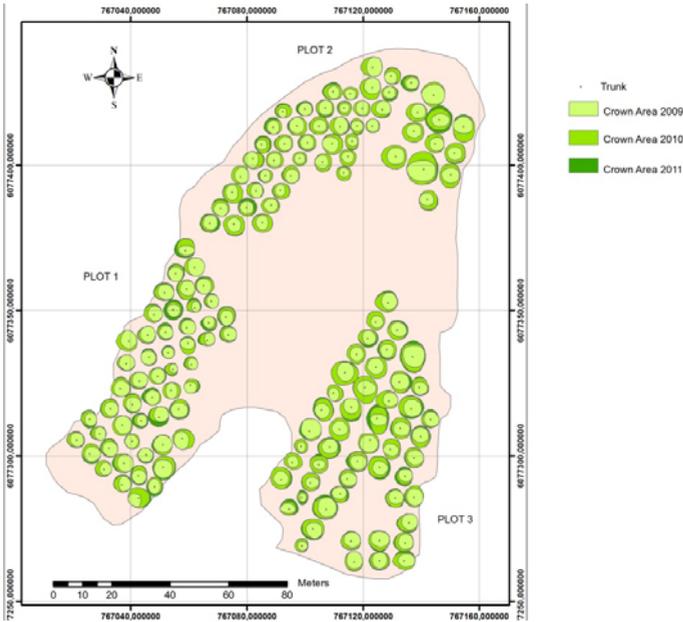


Fig. 4. Thinning Effect on Pencahue stone pine plantation.

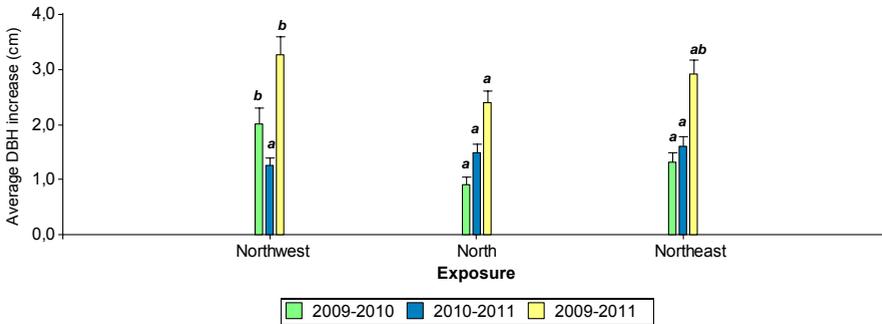


Fig. 5. DBH average increase in Pencahue by exposure. Different letters indicate significant differences between treatments according to Duncan's test ($p \leq 0.05$).

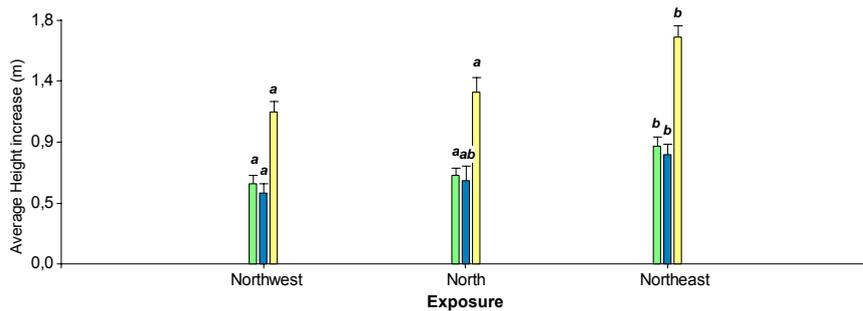


Fig. 6. Height average increase in Pencahue by exposure. Different letters indicate significant differences between treatments according to Duncan's test ($p \leq 0.05$).

IV – Discussion

This study found that thinning produces an increase in crown tree area one and two years after the intervention. Muñoz *et al.* (2008) point out that when removing individuals, the remaining trees have greater access to light, accelerating the growth dynamics in natural forest, generating more biomass. This is coincident with what expressed by Mutke *et al.* (2003), who state that stone pine requires high amounts of light to increase fruit productivity, so when increasing the distance between trees they tend to grow in crown area to intercept more light.

Montero and Candela (1998), conducted a literature review of thinning experiences in Spain, Italy and Portugal, finding great coincidence of several authors in recommending early thinning, between 12 and 15 years old. Following this criterion, interventions performed in both plantations were held at the optimal time limit (15 years in Casablanca and 16 years in Pencahue), so the trees had a rapid response. However, Gordo *et al.* (2009) suggest thinning even earlier, before 10 years. Considering the high vegetative vigor and growth that the species shows in many situations in Chile, we suggest that this option should be tested.

Stone pine has a particular ecological strategy: it shares its biomass in reproductive structures in similar or higher amounts that the one allocated to vegetative growth (Cabanettes *et al.* (1981) cited by Mutke *et al.*, 2007). This strategy was confirmed in this study, since in both plantation plots that showed the highest crown area increase did not exhibit the same tendency for DBH and height parameters, i.e. situations of less vigorous and tree development had a higher DBH increase rather than crown area increase; trees seem to devote their energies to consolidate first, and later to a greater fruit production. That is why the maximum expression of tree growth is found in dominant or isolated trees, whose crowns are not limited and where the strategy of the species is not growing in height but in crown area.

V – Conclusions

We observed a positive effect of thinning in both *P. pinea* plantations one and two years after the intervention; trees tend strategically to maximize space and light, forming rounded and more productive crowns. Although we couldn't assess the thinning influence on fruit production given the duration of the fruit life cycle, we expected it to be positive given the background information on this topic.

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Session 2
**Genetic improvement, selection and
breeding in Mediterranean stone pine**

Mediterranean stone pine (*Pinus pinea* L.) genetic variability for growth traits in a Portuguese provenance trial

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Abstract. Provenance field trials provide information on adaptability, growth and yield of the species. Results from these studies have great practical use in the improvement of forest stands, as it can assure sources of seed that provide well-adapted, productive trees in reforestation and afforestation. The aim of this study was to evaluate Mediterranean stone pine adaptive variability, using a provenance trial initiated in Portugal in 1992. The seed lots were obtained with the cooperation of *Silva Mediterranea* network. The provenance trial included twenty-five seed lots, from seven different countries, collected throughout the species' native range. In February 1993, twenty-five provenances were established at Sines (38°01'N, 8°42'W) and fifteen at Tavira (37°10' N, 7°36' W). Total height and diameter at breast height were used as indicators of provenance variability. Mixed models were applied to data at different ages obtained from those field trials. Results showed that, at least for a significant level of 0.05, provenance genetic variability for height and diameter at breast height was found.

Keywords. *Pinus pinea* L. – Mediterranean stone pine – Genetic variability.

Évaluation de la variabilité génétique du pin parasol (*Pinus pinea* L.) dans un essai de provenance au Portugal

Résumé. Un essai de provenance peut fournir des informations très importantes sur l'adaptabilité, la croissance et le rendement de l'espèce. Les résultats de ces études ont une grande utilité pratique pour l'amélioration des peuplements forestiers, car ils peuvent assurer des sources de graines bien adaptées, en vue du reboisement. Le but de cette étude repose sur l'évaluation de la variabilité du pin parasol vis-à-vis des caractéristiques adaptatives, à travers un essai de provenance établi au Portugal en 1992. Les lots de semences ont été obtenus avec la coopération du réseau Comité *Silva Mediterranea*. L'essai de provenance incluait vingt-cinq lots de graines, originaires de sept pays différents, recueillies dans l'aire de distribution de l'espèce. En février 1993, vingt-cinq provenances ont été plantées à Sines (38°01'N, 8°42'W) et quinze à Tavira (37°10' N, 7°36' W). La hauteur et le diamètre ont été utilisés comme indicateurs de la variabilité entre les provenances. Les modèles mixtes ont été appliqués aux données de hauteur totale à différents âges et aussi au diamètre. Les résultats ont montré que, au moins pour un niveau significatif de 0,05, la variabilité de la hauteur et du diamètre des provenances a été significative.

Mots-clés. *Pinus pinea* L. – Pin parasol – Variabilité génétique.

I – Introduction

Mediterranean stone pine, *Pinus pinea* L., covers about 2.6% of the pines species Mediterranean forest area (Barbéro *et al.*, 1998). In Portugal, this species occupies approximately 118,000 hectares (Fig. 1) which represents 3.5% of the Portuguese total forest area (AFN, 2010). The total area devoted to Mediterranean stone pine has increased since the last inventory of about 51%, which is due to an increased interest in the species by forest land owners (Carrasquinho *et al.*, 2010).

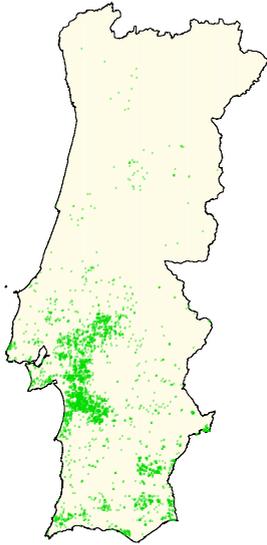


Fig. 1. Distribution of the Mediterranean stone pine (*Pinus pinea* L.) in Portugal (courtesy by Paulo Godinho-Ferreira, AFN, 2010).

Mediterranean stone pine occurs at low and medium altitudes in scattered populations throughout the Mediterranean basin in natural stands or in stands resulting from reforestation activities (Barbéro *et al.*, 1998; Martínez and Montero, 2004). Concerning to its natural area, many authors recognize the difficulty to define it, as this species has been planted for its edible seeds since prehistoric times. Human impact can be expected to have strongly influenced the current distribution of it geographic and genetic diversity (Fady *et al.*, 2004).

According to the Mediterranean Forest Research Agenda (EFIMED, 2009), forest owners and managers need adequate scientifically-based decision support tools for the management of this type of forest to optimize the joint production of multiple goods and services at different temporal and spatial scales in a context of increasing risks. This document, coordinated by the European Forest Institute, describes the main research priorities for the Mediterranean forest, during 2010-2020. One of the four challenges that were identified for the forest sustainability is the impact of climate and land use change on Mediterranean ecosystems. Long-term experiments, such as provenance trials, can be useful for analysing responses of plant material to changing environment. On the other hand, knowing the patterns of genetic variation among populations and proceed on its quantification can provide tools to gene resource management and, in a context of genetic improvement, the raw material for mass selection.

The aim of this study was to evaluate Mediterranean stone pine variability for adaptive traits, such as total height and diameter at breast height, using a provenance trial established in Portugal in 1992.

II – Material and methods

In 1993, National Forest Station, a Portuguese forest research institute, established a provenance trial. In this study, the field sites of *Sines* (38°01'N, 8°42'W) and *Tavira* (37°10'N, 7°36'W) were evaluated.

Twenty five seed lots, from seven different countries, Portugal, Spain, Italy, Greece, Morocco, Israel and Turkey, were obtained throughout the species' native range, with the cooperation of Silva Mediterranea network. Table 1 indicates the location and climate information of these provenances seed lots that are represented in the field sites. The provenances are considered to be a sample from different ecological conditions (latitude, longitude, altitude, rainfall and temperature). The altitude ranges from sea level to 1007 meters (seed lot from Spain, Cordillera Central). The lower annual precipitations are 324 mm, 373 mm and 411 mm from Spain, Sierra Morena (S₂₆) and Cordillera Central (S₂₇) and Greece, Mandrakii (G₃), respectively. Ponte de Lima (P₉), Vieira do Minho (P₁₀), and Amarante (P₁₁), three locations from the north of Portugal, correspond to the higher mean annual precipitation.

Twenty-five and fifteen provenances were established at *Sines* and *Tavira* field sites, respectively (Carneiro *et al.*, 2000a). Unequal distribution of seed sources within the two experimental sites was related to different seed germination per seed lot.

The two field sites were established as a randomized complete block design with six replicates for each provenance. Twenty-five seedlings per plot (5 x 5) were planted at a spacing of 3 x 4 meters, within and between rows, respectively.

Growth traits, total height (h_n) and diameter at breast height (d_{13}), were used for evaluation of provenance genetic variability. In *Sines* field site, h_n (cm) was assessed at age (n) two, four, five, six, ten and eleven, and d_{13} (centimeters) at age thirteen, for each individual tree. In *Tavira*, total height was assessed at age two, four, five, and six. To avoid border effects, the border line trees of the trial were not considered in data analysis.

The methodology for statistical analysis followed a linear mixed model, using MIXED Procedure of SAS version 9.2 (SAS Institute Inc. 2008). Single tree data of total height (h_n) and diameter at breast height (d_{13}) were used. The analysis was performed assuming fixed effects for the overall mean and block effects, and random effects for provenances and plots. Spatial correlated random errors were modelled according to a power correlation function and different correlations for row and column directions were assumed. This is equivalent to a separable first order autoregressive model (Gilmour *et al.* 1997). When necessary, an independent error term was also added. Model parameters were estimated by residual maximum likelihood method (REML, Patterson and Thompson 1971), using the Fisher-Scoring algorithm (Jennrich and Sampson, 1976).

A residual likelihood ratio test (REMLRT) was used to test the provenance genetic variance component associated to each trait. Because the null hypothesis (provenance genetic variance equal to zero) specifies a value on the boundary of the parameter space, the p-value was assumed to be half of the reported p-value from a chi-squared distribution with one degree of freedom (Self and Liang, 1987 and Stram and Lee, 1994).

For a better comparison of provenance variability among traits and between sites, the coefficient of genetic provenance variation was computed (ratio between the estimates for the provenance genetic standard deviation and the estimates for the overall mean).

Following the mixed model equations (Henderson, 1975), the empirical best linear unbiased predictor (EBLUP) of genotypic effect for the total height at age six (the maximum common age for both field sites) was obtained for each provenance. Provenance performance evaluation was then based on the ranking of the predicted height at age six (EBLUP of the provenance genetic effects plus overall mean estimate).

Table 1. Location and climate information for *Pinus pinea* L. provenances represented in Sines and Tavira field trials

Code	Provenance name	Country	Latitude (N)	Longitude	Altitude (m)	Mean precipitation (mm)			Mean temperature (°C)		
						Annual	Driest month	Wettest month	Annual	Warmest month	Coldest month
G2	Strophilia	Greece	38°08'	21°22' E	5	673.2	1.2	129.2	16.8	24.9	9.6
G3	Mandraki	Greece	39°10'	23°24' E	24	411.3	2.8	60.5	15.6	25.0	7.2
Is8	Monte Carmelo1	Israel	32°45'	35° E	300	490.2	0	139.5	17.1	26.4	11.6
Is13	Monte Carmelo2	Israel	32°45'	35° E	400	490.2	0	139.5	17.1	26.4	11.6
It4	Cecina	Italy	43°45'	10°18' E	4	774.1	18.3	104.9	14.7	23.2	7.0
It6	Tomboli di Cecina	Italy	43°09'	11°17' E	300	591.6	20.5	82.8	13.7	22.6	5.9
It18	Duna Felicia	Italy	42°02'	12°27' E	70	684.2	11.9	101.2	15.2	23.7	7.5
M1	Koudia Hamra	Morocco	35°11'	6°10'10" W	650	545.5	0	100.0	17.3	22.8	12.2
M7	Ain Grana	Morocco	35°16'50"	5°53'15" W	130	565.7	0.1	101.0	17.3	23.2	12.1
M14	Cap Spartel	Morocco	35°47'16"	5°53'11" W	220	573.6	0.1	107.4	17.5	23.5	12.4
M20	Dunes D' Adjir	Morocco	35°12'21"	5°53'47" W	50	540.4	0	95.9	17.6	23.4	12.4
P5	Alcácer do Sal	Portugal	38°15'	8°30' W	75	500.2	0.2	75.7	16.9	23.2	11.2
P9	Ponte de Lima	Portugal	41°46'	8°36' W	300	1317.4	15.6	194.6	13.9	19.6	8.6
P10	Vieira do Minho	Portugal	41°41'	8°06' W	750	1256.8	15.9	180.8	11.8	18.9	5.7
P11	Amarante	Portugal	41°18'	8°06' W	245	1035.8	9.5	148.0	13.7	20.4	7.7
P22	Viseu	Portugal	40°40'	7°54' W	420	919.5	5.8	131.1	14.3	21.3	8.1
S25	Andalucia	Spain	36°20'	6°05' W	50	514.1	0.1	97.4	17.8	24.1	12.3
S26	Sierra Morena	Spain	38°10'	4°00' W	500	372.9	0.7	50.4	17.0	27.1	8.8
S27	CordilleraCentral	Spain	40°30'	4°20' W	1007	324.2	5.4	40.5	13.1	23.1	5.2
Tk12	Yatagan-katrancı	Turkey	37°27'	27°55' E	600	737.2	3.6	142.5	14.7	24.8	5.6
Tk15	Yalova	Turkey	48°32'38"	29°22'49" E	500	629.4	15.0	102.1	13.5	22.1	4.7
Tk16	Aydin-Karine	Turkey	37°46'00"	27°23' E	450	620.6	2.3	142.1	16.4	25.5	8.2
Tk17	Kumluca	Turkey	36°17'45"	30°20'02" E	5	750.2	1.1	177.8	17.4	26.1	9.7
Tk19	Serik	Turkey	36°52'05"	31°01'15" E	10	514.0	6.3	81.3	10.1	20.6	-0.2
Tk21	Çanakkale	Turkey	40°19'36"	26°16'31" E	20	535.6	3.9	85.3	14.8	24.0	6.0

Note: Climate data were obtained from the International Water Management Institute (<http://wcatlas.iwmi.org/results.asp>) using latitude and Longitude data.

III – Results and discussion

As it can be observed, in Table 2, the REMLRT for the provenance genetic variance component indicates that the provenance genetic variability was always significant (p -value<0.05) for total height at all ages, in both field sites and also for diameter at breast height in *Sines* at age 13.

Table 2. Estimates for the overall mean and for the provenance genetic variance ($\hat{\sigma}_{prov}^2$), residual likelihood ratio test (REMLRT) for the provenance genetic variance component, and coefficient of genetic variation for provenances (CV_{prov}) for the traits h_n and d_{13} and for the two field sites: *Sines* and *Tavira*

Trait	Overall mean estimate (cm)	$\hat{\sigma}_{prov}^2$	$-2l_R$	$-2l_{R0}$	REMLRT		CV_{prov} (%)
					D	p-value	
Sines							
h_2	27.40	2.95	23200.3	23216.9	16.6	<0.0001	6.27
h_4	69.00	12.55	29303.2	29309.6	6.4	0.0057	5.13
h_5	91.55	34.11	30499.2	30504.1	4.9	0.0134	6.38
h_6	129.60	62.11	32326.8	32331.2	4.4	0.0180	6.08
h_{10}	217.13	184.65	34409.4	34416.9	7.5	0.0031	6.26
h_{11}	239.07	233.92	34935.5	34943.4	7.9	0.0025	6.40
d_{13}	7.72	0.35	14909.6	14943.9	9.3	0.0011	7.68
Tavira							
h_2	34.55	1.48	13076.8	13082.7	5.9	0.0076	3.52
h_4	92.13	8.97	15643.0	15650.5	7.5	0.0031	3.25
h_5	110.62	22.76	16167.8	16181.7	13.9	<0.0001	4.31
h_6	136.69	35.68	18697.0	17027.5	9.7	0.0009	4.37

Note: $-2l_R$ and $-2l_{R0}$ are the minus twice the residual log-likelihood for the full model and for its variant without provenance effect, respectively; $D=[(-2l_{R0})-(-2l_R)]$.

Carneiro *et al.* (2006) found significant differences among provenances for total height at ages 2 and 13, but not for diameter at breast height at age 13. However, these authors fitted a classic model, assuming fixed effects for provenances and independent and identically distributed errors. In addition, only the central trees inside each provenance plot were considered and the plot factor was not included.

The studies concerning *Pinus pinea* L. variability are few and contradictory (Carneiro *et al.*, 2006; Mutke *et al.*, 2010). It seems that, the variability evaluation for *Pinus pinea* L. using provenance trials depends on the methodologies of data analysis.

Comparing the two field sites, at the same ages, we verified that, for each age, the overall mean height at *Tavira* was always higher than at *Sines*. The ecological conditions at *Tavira* promoted the stone pine growth, as it was also verified by Carneiro *et al.* (2000b). They have evaluated flowering and fructification at age five, in *Tavira* and *Sines* field sites and concluded that the amount of the flowers and the cones were the double at *Tavira*.

Considering the coefficient of genetic variation for provenances, for total heights at different ages, the values ranged from 5.13% to 6.40% in *Sines* and from 3.25% to 4.37% in *Tavira*. That is, the higher values for the CV_{prov} were obtained for *Sines*, as a larger number of provenances, 25, were used in this field site, while in *Tavira*, only 15 were studied. Therefore, it is reasonable to state that with larger number of provenances becomes easier to detect variability among them.

For each of the twenty-five provenances from the Sines field site and the fifteen from the Tavira site, the predicted height at age 6 are shown in Table 3.

Table 3. Predicted height (cm), at age six, of the provenances and their respective rankings in the two field sites: Sines and Tavira

Provenance code	Predicted height (cm)			
	Sines	Rank	Tavira	Rank
G2	124.10	22	125.97	15
G3	121.90	24		
Is8	123.96	23		
Is13	137.30	4		
It4	137.92	3	142.82	2
It6	128.61	15	138.44	6
It18	138.63	2	145.72	1
M1	140.29	1	142.41	3
M7	128.39	16	139.48	4
M14	124.43	21		
M20	133.79	5		
P5	129.40	14	133.60	12
P9	130.11	12		
P10	130.53	11		
P11	129.65	13		
P22	124.77	20	134.09	10
S25	126.81	17		
S26	131.14	9	135.98	8
S27	130.63	10		
Tk12	119.32	25	134.68	9
Tk15	131.31	8	137.75	7
Tk16	132.44	7	133.27	14
Tk17	124.95	19	133.47	13
Tk19	126.24	18	133.61	11
Tk21	133.35	6	139.05	5

At Sines field site, it can be observed that the Moroccan provenance M1, the two Italians provenances It18 and It4, the Israeli provenance Is13, and the Moroccan provenance M20, were, respectively, the top five provenances. The Portuguese provenances P5, P9, P10 and P11 performances were around the overall mean estimate (129.60 cm), but the P22 fell below this mean estimate. The Turkish provenances Tk17 and Tk19, displayed similar behavior as the latter Portuguese provenance. The Turkish provenance Tk12, the Greek provenances G3 and G2, and the Israeli provenance Is8 showed a low growth performance. At the Tavira field site, provenances It18, It4, M1 and M7 were also found to be in the top five of the ranking. On the contrary, Tk16 and Tk17 were among the lowest performers. As it happened in Sines, the Greek provenance G2 ranked the lowest also in Tavira. The only Portuguese provenance included in this site, P22, showed the same tendency as in Sines (below the overall mean estimate, 136.69 cm).

The common assumption that local seed sources will outperform other provenance of the same species (Zobel and Talbert, 1984) was not verified in this study, for Sines field site. The

performance of the local seed source, P5, was lower than the performance of foreign provenances.

IV – Conclusions

Provenance variability for growth traits was detected in both field sites, *Sines* and *Tavira*. However, since genetic variability results on *Pinus pinea* L. are inconsistent among the available studies, it is strongly advised to improve research on statistical models for this type of data.

The existence of provenance variability for growth traits permits not only to perform mass selection, but also to hope to detect genetic variability in other economically important traits, such as, kernel production and/or kernel quality.

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Provenance trials of stone pine (*Pinus pinea* L.) in the Aegean region: Tenth year evaluation

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Abstract. The purpose of the project here analysed was the establishment of provenance trials in natural environment of stone pines in order to determine adaptation ability and growth performance as well as to conserve gene materials of various origins in ex-situ environments. This project has been simultaneously implemented in France, Italy, Spain, Tunisia, Morocco and Turkey under the coordination of FAO-INRA. A total of 26 origins have been used in the trial with three replications in Kozak and Yigintas plots of the Aegean Region of Turkey. Spacing for the saplings was 6 × 6 m at both of the plots. Because no significant differences were found at the statistical analysis of 5th year data, here the collected data after 10 years were analyzed and evaluated. According to the variance analysis, significant differences were found between the provenances in terms of mean height (p value 0.1 %), mean diameter (0.5 %) and mean number of conelets (0.1 %). However, there were not any significant differences among the crown diameters and the survival ratios. There were significant differences between the trial plots. The reasons of the differences between two trial plots might be the distance to the sea, bedrock type and soil depth. For more precise evaluation of the provenance trials, the tree age should be minimum 20 years, therefore we should wait for results from next decade.

Keywords. Aegean Region – *Pinus pinea* – origin.

Essais de provenance du pin pignon (*Pinus pinea* L.) dans la région égéenne : évaluation au bout de dix ans

Résumé. Le but du projet était d'analyser la mise en place d'essais de provenance dans un environnement naturel de pins pignons afin de déterminer la capacité d'adaptation et de croissance et de préserver un matériel génétique d'origine diverse dans des environnements ex situ. Ce projet a été mis en œuvre simultanément en France, en Italie, en Espagne, en Tunisie, au Maroc et en Turquie sous la coordination de la FAO-INRA. Un total de 26 origines ont été utilisées dans le processus avec trois répétitions dans les parcelles de Kozak et Yigintas de la région égéenne de la Turquie. L'espacement des plants était de 6 × 6 m dans les deux parcelles. Vu qu'aucune différence significative n'avait été trouvée pour l'analyse statistique des données de 5^{ème} année, nous présentons les données recueillies après 10 ans, qui ont été analysées et évaluées. Selon l'analyse de variance, des différences significatives ont été trouvées entre les provenances en termes de hauteur moyenne (valeur p 0,1%), de diamètre moyen (0,5%) et de nombre moyen de jeunes cônes (0,1%). Cependant, il n'y avait pas de différences significatives entre les diamètres de la couronne et les ratios de survie. Il y avait des différences significatives entre les parcelles d'essais. Les raisons des différences entre les deux parcelles d'essai pourraient être la distance à la mer, le type de roche et la profondeur du sol.

Mots-clés. Région égéenne – *Pinus pinea* – origine.

I – Introduction

The interest of stone pine is due to its seeds rather than its wood. Approximately 1,200 tons of nuts per year are produced in Turkey, and average 1,000 tons of this production are provided by Aegean region. Bilgin and Ay (1997) determined the mean nut yield in between 68-118 kg/ha in this region, versus some 200 kg/ha in Spain.

According to Urgenc (1982), provenance trials are important in order to identify the most suitable origins for the certain regions. Therefore, the determination of the adaptation and the

seed ability of different stone pine origins tested in various ecological regions in Turkey is going to facilitate the management of economic resources realistically.

Even though there are other origin trial studies conducted on stone pine species in Turkey, the current study is the only one that includes also stone pine provenances from the other countries. As a result of the study, it is aimed to determine the adaptation abilities and the growth performances of the origins, to ex-situ conserve the gene resources of the origins and to identify the cone yields of the origins.

II – Material

A total of 26 origins, 11 native Turkish (Fig. 1) and 15 from other countries (Table 1), have been used in the trial with three replications in Kozak (Izmir, Bergama) and Yigintas (Aydın, Koçarlı) plots of the Aegean Region of Turkey. Latitude of Kozak is 39° 16' 21" N, longitude is 27° 09' 32" E. Koçarlı latitude is 37° 39' 44" N, longitude is 27° 40' 22" E'. Spacing for the saplings was 6 × 6 m in both plots.

In each trial, tree height and stem diameter at breast height were measured at five and ten years, and the cone number per tree was registered.



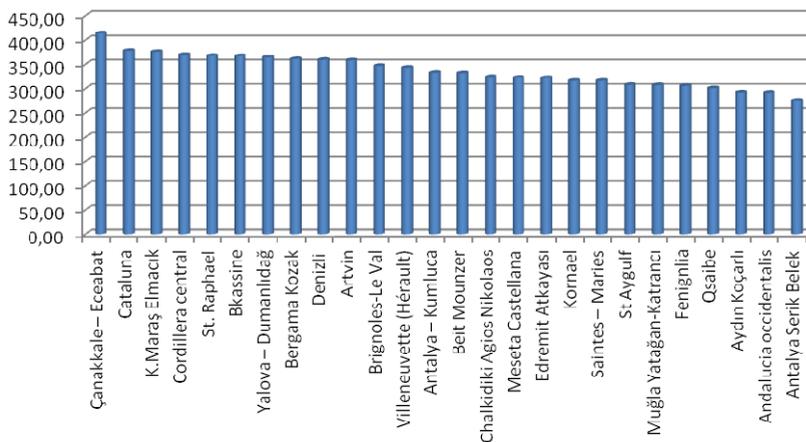
Fig. 1. Distribution areas of stone pine in Turkey.

III – Results

According to the data analysis at 10 years (Box *et al.*, 1997), in Kozak, the provenances that have the maximum mean height were Eceabat/TR ($h_{\text{mean}}=414$ cm), Cataluña/SP ($h_{\text{mean}}=378$ cm) and Elmacı/TR ($h_{\text{mean}}=376$ cm) (Fig. 2). The mean height and also mean diameter of Serik/TR ($h_{\text{mean}}=275$ cm, $d_{\text{mean}}=5.7$ cm) was the minimum at this plot. The provenances with the maximum mean diameter were Cataluña/SP ($d_{\text{mean}}=15.1$ cm), Villeneuve (Hérault) ($d_{\text{mean}}=12.8$ cm and Saintes-Maries /FR ($d_{\text{mean}}=12.6$ cm) (Fig.3). St. Raphael/FR ($nc_{\text{mean}}=6.5$) and Artvin/TR ($nc_{\text{mean}}=6.1$) provenances has showed the best performance in terms of mean number of conelets, while the mean number of the conelets of Yatağan/TR provenance was the minimum ($nc_{\text{mean}}=2.9$) (Fig. 4).

Table 1. Provenances used in the trials

Number	Provenance	Country	Altitude (m)	Rainfall (mm)
1	Brignoles-Le Val	France	310	813
2	Saintes-Maries	France	2	543
3	Bkassine	Lebanon	800	-
4	St Aygulf	France	10	943
5	Fenignlia	Italy	25	680
6	Andalucia occidental	Spain	50	631
7	Chalkidiki Agios Nikolaos	Greece	50-400	439
8	Beit Mounzer	Lebanon	1300-1400	-
9	St. Raphael	France	80	943
10	Cordillera central	Spain	900	1,007
11	Muğla Yatağan-Katrançı	Turkey	685	752
12	Çanakkale Eceabat	Turkey	20	629
13	Denizli	Turkey	650	-
14	Antalya Kumluca	Turkey	5	986
15	Qsaibe	Lebanon	600-700	-
16	Meseta Castellana	Spain	875	452
17	Cataluna	Spain	150	710
18	Kornael	Lebanon	1200-1400	-
19	Villeneuve (Hérault)	France	130-220	775
20	Bergama Kozak	Turkey	460	668
21	Antalya Serik Belek	Turkey	5	1,030
22	Edremit Atkayası	Turkey	415	733
23	Yalova Dumanlıdağ	Turkey	500	725
24	Aydın Koçarlı	Turkey	450	-
25	Artvin	Turkey	250	1,009
26	K.Maraş Elmacık	Turkey	750	1,151

**Fig. 2. Average tree height of provenances at Kozak (cm).**

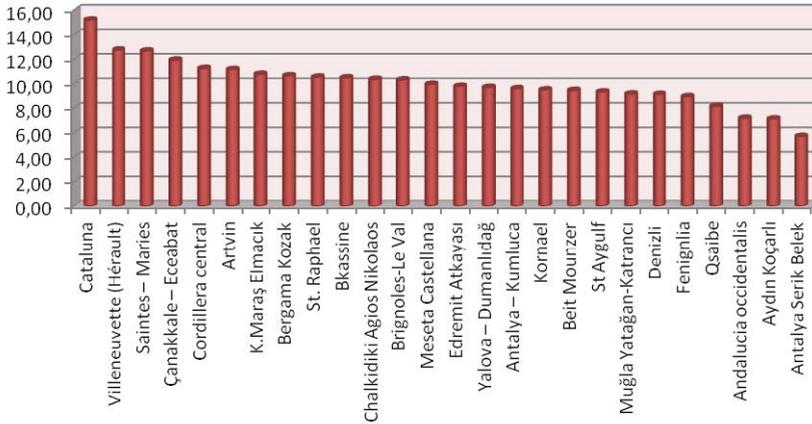


Fig. 3. Average stem diameter of provenances at Kozak (cm).

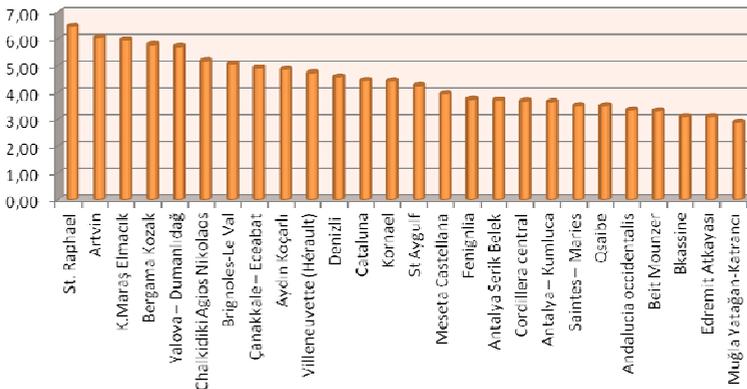


Fig. 4. Average conelet number of provenances at Kozak.

On the other hand, according to the data analysis at 10 years in Koçarlı, the provenances that have the maximum mean height were Yatağan/TR ($h_{\text{mean}}=357$ cm, $d_{\text{mean}}=11.5$ cm), Brignoles/FR ($h_{\text{mean}}=342$ cm, $d_{\text{mean}}=8.6$ cm) and St Aygulf/FR ($h_{\text{mean}}=312$ cm, $d_{\text{mean}}=8.5$ cm). These provenances showed also the maximum mean diameter. The mean height of Chalkidiki/GR ($h_{\text{mean}}=198$ cm) was the minimum while the mean diameter of Kumluca/TR ($d_{\text{mean}}=3.1$ cm) was the minimum at this plot. Yatağan/TR ($nc_{\text{mean}}= 5.4$) and Saintes Maries/FR ($nc_{\text{mean}}= 4.5$) showed highest number of conelets, while still no tree of Cataluna/SP produced any conelets at all (Figs 5, 6 and 7).

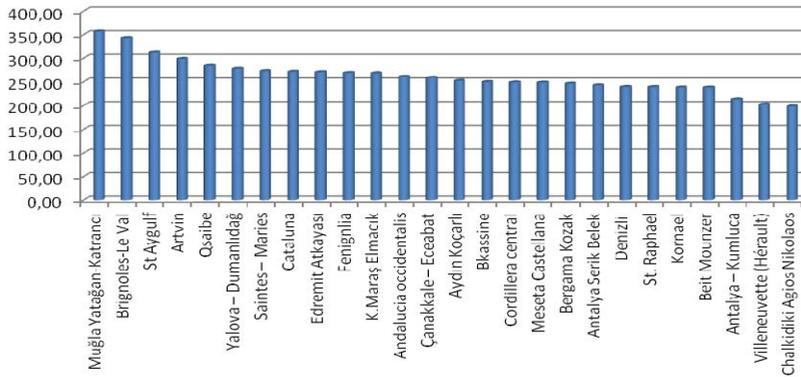


Fig. 5. Average tree height of provenances at Koçarlı (cm).

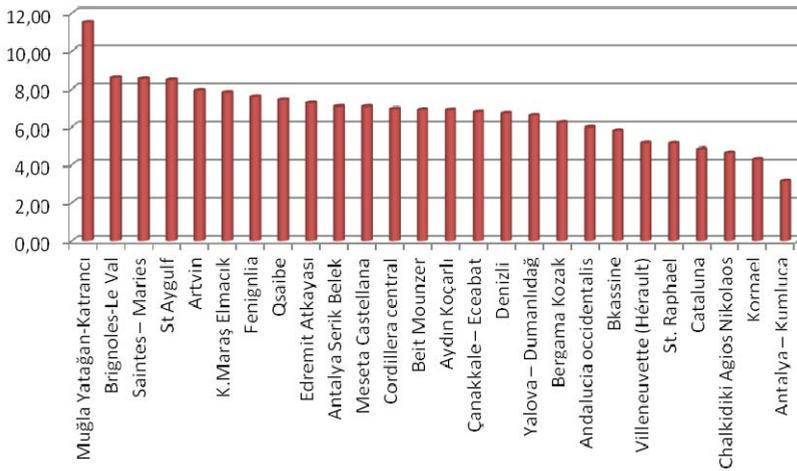


Fig. 6. Average stem diameter of provenances at Koçarlı (cm).

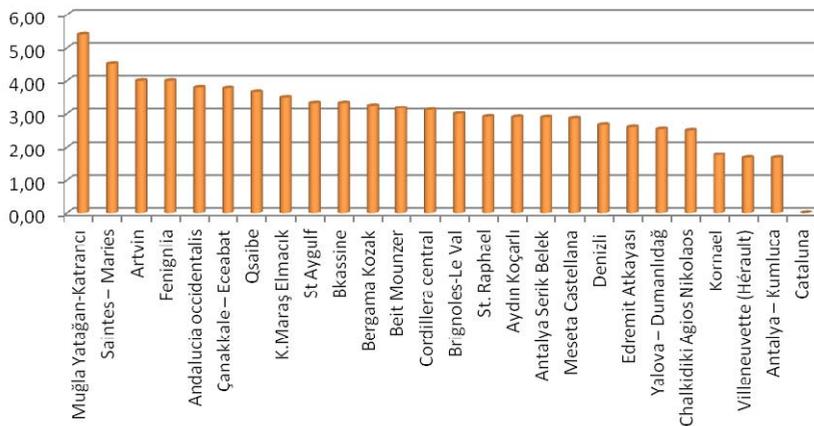


Fig. 7. Average conelet number of provenances at Koçarlı.

IV – Discussion

The main purpose of afforestation with stone pine in Turkey is the pine nut production. For this reason, it is important to determine the origins possessing high pine nut yield. Therefore, not only height and crown diameter but also the number of mature cones and seed numbers in those cones, seed weights and sizes, seed quality etc. parameters should be monitored in the future. Additionally, because crown diameter is important in increasing in cone yield, there is a need for thinning after the 10th year, in order to provide an adequate growth space. In this study, Turkish origins are seen to be more successful than foreign origins, but for more precise evaluation of the provenance trials, the tree age should be minimum 20 (at least the half of the rotation period). Therefore we should wait for results from next decade.

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Low genetic and high environmental diversity at adaptive traits in *Pinus pinea* from provenance tests in France and Spain

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Abstract. Mediterranean Stone pine (*Pinus pinea* L.) is a native species of the Mediterranean region, widely used for reforestation. It is characterised by a low genetic variation both at phenotypic traits and molecular markers. In the early nineties, experimental plots were established in several countries bordering the Mediterranean Sea for studying the distribution of genetic variability among provenances in adaptive traits as survival, vegetative and reproductive phase change, phenology and growth. Here we report results of the first decade at seven trial sites in France and Spain. The survival, ontogeny and growth patterns were homogeneous among provenances but differed among sites. On the other hand, the phenotypic plasticity of the species and its sensitivity to microsite (soil) variation produced strong spatial autocorrelations of the response variables, both between and within sites, which masked greatly the variation between provenances. Nearest-neighbours adjustments that captured the spatial pattern at each site reduced the error term and increased the significance of the geographic genetic variation considerably. Comparison of the adjusted average heights of each provenance showed a common trend between sites, with a lightly more vigorous growth in plants from colder or inland provenances. These results confirm the conclusions obtained in earlier analyses of the Spanish test sites, where these provenances showed also an earlier spring shoot phenology and a stronger tendency to polycyclism.

Keywords. *Pinus pinea* – Common garden experiments – Phenotypic plasticity – Nearest neighbours adjustment – Iterative spatial analysis.

Faible variabilité génétique et forte variabilité environnementale des caractères adaptatifs de *Pinus pinea* en plantation comparative en France et en Espagne

Résumé. Le pin pignon (*Pinus pinea* L.) est un arbre forestier endémique du bassin méditerranéen, largement utilisé en reboisement. Il se caractérise par une faible diversité génétique de ses caractères morphologiques et une faible variabilité génétique au niveau des marqueurs moléculaires. Au début des années 1990, plusieurs tests de provenance ont été installés conjointement dans plusieurs pays méditerranéens pour évaluer la structure de la diversité génétique de caractères importants pour l'adaptation : survie, changements ontologiques entre les phases végétatives et reproductives, phénologie et croissance. Dans cet article, nous présentons les résultats d'analyses de la variabilité de ces caractères à l'issue d'une décennie de croissance dans 7 des tests de provenance installés en France et en Espagne. Les différentes provenances ne montrent pas de différences significatives pour la survie, ni pour l'ontogénie de la croissance, alors que les différences sont fortes entre sites. Les génotypes montrent une forte plasticité phénotypique en réponse aux variations de sol entre différents sites et à l'intérieur des sites, qui masque la variabilité entre provenances. Les ajustements aux plus proches voisins qui permettent de prendre en compte les effets spatiaux à l'intérieur des sites ont permis de mettre en évidence une diversité génétique significative entre provenances. La comparaison des moyennes de provenance ajustées montre une croissance plus vigoureuse des provenances issues des sites les plus froids et les plus intérieurs. Ces résultats confirment des résultats plus anciens issus des seuls tests de provenance espagnols, dans lesquels ces provenances montraient un débourrement printanier plus précoce et une tendance plus marquée au polycyclisme.

I – Introduction

Mediterranean Stone pine (*Pinus pinea* L.) is a native species widely used for protective or restoring afforestations in the Mediterranean region, and occasionally in other similar climate zones around the world, aiming soil protection, ecosystem restoration and sustainable production of both pine nut and timber. Its area around the Mediterranean Sea has more than doubled in the last two centuries as results of these reforestations, stone pine-dominated forests occupying currently about 0.75 million hectares, sparsely spread from the Atlantic coast in Portugal to the shores of the Black Sea and the Mount Lebanon, and also at the southern shore of the Mediterranean Sea, where it is supposed to have been introduced only in the XX century, as well as in other bioregions with similar climates, such as California, Chile, South Africa, or Australia. In other climate zones like northern Iran or even Ethiopia, and in temperate areas like Great Britain, New Zealand, or Argentina, stone pine is an exotic restricted mainly to ornamental uses in gardening, though in some regions its growth outperforms greatly the original Mediterranean populations on semiarid or dry Mediterranean sites, reaching dominant heights up to 35 m (Mutke *et al.*, 2012).

Though since Antiquity, the stone pine has been a cultural species in the Mediterranean due to its ornamental value and its edible kernels, up to present most of its stands are natural or naturalised forests or rural groves, not horticultural plantations based on defined cultivars. However, no phylogenetic structure has been found within or among its widely separated, most ancient populations, because all of them are fixed to a same haplotype, at least for nearly all studied cpDNA and allozyme markers (Fallour *et al.*, 1997; Vendramin *et al.*, 2008). This near absence of genetic variation for molecular markers among populations, very rare for a widespread forest tree species, has also been observed in phenotypic traits and is attributed to its putative human spread by seed transfer from a narrow source population (Court-Picon *et al.*, 2004; Gordo *et al.*, 2007). It might be also related to its strong phenotypic plasticity that allows for a high adaptative potential in spite of a low genetic diversity, e.g. resulting in high survival rates even in severe environments, though with strong ontogenic delays as pay-off, such as retarded growth or phase changes (Mutke *et al.*, 2010). In view of on-going and further expected climatic changes, the relevance of both plasticity and genetic diversity for the adaptive potential of forest species is a current issue of research, as well as the future risks of population decline or extinction at the rear edge of species' distribution under changing conditions (Matyas *et al.*, 2009). In this context, Mediterranean stone pine might be an interesting model species for studying the role of phenotypic plasticity (and e.g. its epigenetic mechanisms) when genetic differentiation is nearly absent.

In the early nineties, as cooperation within the FAO framework *Silva Mediterranea* and coordinated by French INRA, *Pinus pinea* seed lots were exchanged and experimental plots of a reciprocal provenance test were established in several countries bordering the Mediterranean Sea. The objective was to study the genetic variability between provenances in adaptative traits such as survival, phenology or growth. Several preliminary results have been published already for the test sites in France (Court-Picon *et al.*, 2004), Portugal (Carneiro *et al.*, 2006), Spain (Mutke *et al.*, 2010), Maroc (Sbay, 2000), and Tunisia (Khaldi *et al.*, 2009), and current results for Portuguese and Turkish test sites are presented in this issue of *Options Méditerranéennes* (Carrasquinho and Gonçalves, 2012; Acar *et al.*, 2012). The present communication puts together comparative results of the first fifteen years at seven trial sites in France and Spain.

II – Materials and methods

The tested accessions correspond to 40 provenances from Lebanon, Turkey, Greece, Italy, France, Spain, Portugal, Maroc, and Tunisia (Fig. 1), though only eight provenances are represented at all seven analysed sites (three in France and four in Spain), whereas other seven accessions (hence summing up fifteen) are present at least in five test sites in both countries (Table 1).

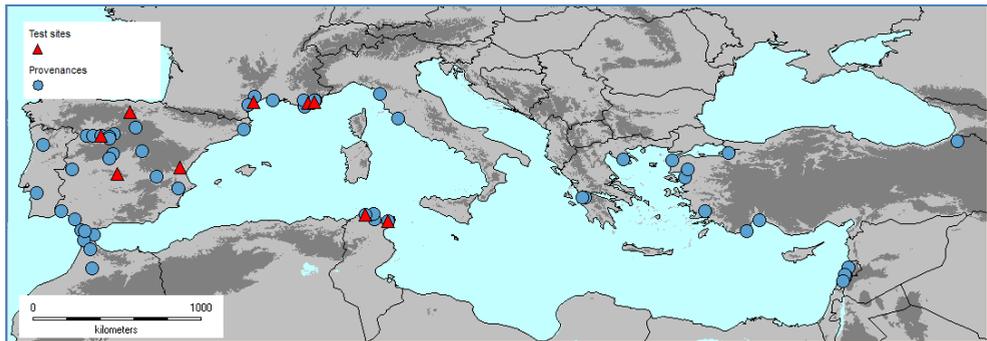


Fig. 1. Mediterranean stone pine provenances and analysed test sites of the provenance trial (also the discussed two Tunisian test sites are included in the map).

The repeated measurements at each site included the survival, occurrence of vegetative and reproductive phase changes, phenology, height and diameter. Given that no significant differences in survival or ontogeny were found between accessions, the present analysis centres on height growth.

Due to the presence of strong spatial autocorrelation (microsite pattern in mosaic) at the French and Spanish test sites, where the spatial individual tree position (coordinates) was included in the dataset, the analysis of variance adjusting a general linear model integrated an Iterative Spatial Analysis (ISA). For this, a Nearest Neighbourhood adjustment (NNA) used iteratively the mean residual values for the eight adjacent plant positions as covariate proxy for estimating a local microsite effect for each tree, following the methods used in the previous work on former measurements of the Spanish test sites (Mutke *et al.*, 2010) and on other genetic field tests (cf. Mutke *et al.*, 2007).

Planting and measurement dates differed between test sites, so only the most recent height at each site were analysed for this study. In Spain, test sites Tordesillas and Trespaderne were measured last in 2007 at age 13 years, Quintos de Mora in 2008 (14 yrs.), whereas Cucalón has not been re-measured since 2003 (9 yrs.; Mutke *et al.*, 2010). In France, test site La Gaillarde was measured last in 2002 (8 yrs.), Sainte Baume in 2003 (10 yrs.) and Le Treps in 2010 (16 yrs.).

The adjusted provenance mean values at each site were compared in a Principal Component Analysis, including also the provenances mean values estimated at two Tunisian plots, Jbel Essmaa and Oued El Bir, at age 9 and 10 by Khaldi *et al.* (2009) adjusting a general linear model without spatial analysis.

Table 1. 34 accessions. mean annual temperature and rainfall and presence at the seven test sites and at two Tunisian sites

Provenance	Country	MAT	MAR	CUC	TOR	TRE	QUI	LT	StB	LG	TN
Chalkidiki Agios Nic	GR	15.0	439	1	1	1	1	1	1	1	1
Feniglia 67	I	15.8	680	1	1	-	-	1	1	1	1
Beit Mounzer (Mad. J.)	RL	13.3	1030	1	1	-	-	1	1	1	1
Kornaele	RL	13.2	1148	1	1	1	1	1	1	1	-
Qsaibe	RL	17.1	1098	1	1	-	-	1	1	1	1
Bkassine	RL	14.3	977	1	1	-	-	1	1	1	1
Antalya-Kumluca-Kalm.	TR	18.6	1030	1	1	1	1	1	1	1	1
Yalova-Dumanlidag	TR	11.7	754	1	1	1	1	1	-	1	-
Canakkale-Eceabat	TR	15.0	626	1	1	1	1	1	1	1	1
Mugla-Yatagan-Katran	TR	16.3	1202	1	1	1	1	1	1	1	1
St. Raphaël (Var)	F	14.0	848	1	1	1	1	1	-	1	1
Villeneuve (Hér.)	F	13.5	775	1	1	1	1	1	1	1	1
Saintes-Maries	F	14.3	543	1	1	1	1	1	1	1	1
St. Aygulf (Var)	F	14.6	848	1	1	1	1	1	-	1	1
Brignoles- Le Val	F	13.1	813	1	1	1	1	1	1	1	1
Alcacer do Sal	P	16.3	589	1	1	1	1	-	-	-	-
Serra do Minho	P	14.0	1296	1	1	1	1	-	-	-	-
Cogeces de Iscar (VA)	E	12.2	455	1	1	1	1	-	-	-	-
Valorio, fondo (ZA)	E	12.3	359	1	1	1	1	-	-	-	-
Valorio, ladera (ZA)	E	12.3	359	1	1	1	1	-	-	-	-
Toro (ZA)	E	12.0	366	1	1	1	1	-	-	-	-
Vega Sicilia (VA)	E	11.3	461	1	1	1	1	-	-	-	-
Tordesillas (VA)	E	12.0	432	1	1	1	1	-	-	-	-
Portillo (VA)	E	11.6	470	1	1	1	1	-	-	-	-
Íscar (VA)	E	12.2	440	1	1	1	1	-	-	-	-
Cadalso (M)	E	13.6	687	1	1	1	1	-	-	-	-
Budia (GU)	E	12.9	530	1	1	-	-	-	-	-	-
Biar (A)	E	12.9	457	1	1	1	1	-	-	-	-
Tarazona de la Mancha	E	13.4	453	1	1	1	1	-	-	-	-
Parafrugell (GI)	E	15.3	665	1	1	1	1	-	-	-	-
Garrovillas (CC)	E	16.4	574	1	1	1	1	-	-	-	-
Doñana (CA)	E	17.5	575	1	1	1	1	-	-	-	-
Las Lomas (CA)	E	17.9	550	1	1	1	1	-	-	-	-
Cartaya (H)	E	18.1	500	1	1	1	1	-	-	-	-

MAT mean annual temperature; MAR mean annual rainfall; Presence at test sites: CUC Cucalón (Spain); TOR Tordesillas (Spain); TRE Trepaderne (Spain); QUI Quintos de Mora (Spain); LT Le Treps (France); StB Sainte Baume (France); LG La Gaillarde (France); TN Tunisia (Khaldi *et al.*, 2009).

III – Results and discussion

Height growth differed significantly between provenances, though this genetic variability (at each sites, 3-12% of the phenotypic variance) was quite minor than the common, stable reaction norm in dependence on site (among trials) and microsite, which produced strong spatial autocorrelations of tree height that masked those genetic differences.

Table 2. Adjusted mean height (cm) of 34 accessions at the test sites where they are present

Provenance	CUC	TOR	TRE	QUI	LT	StB	LG	Tun
Chalkidiki Agios Nic	173	468	107	200	390	142	142	196
Feniglia 67	228	482	-	-	420	156	142	213
Beit Mounzer (Mad. J)	198	468	-	-	400	159	153	214
Kornaele	190	441	100	196	405	145	135	-
Qsaibe	192	443	-	-	395	153	144	200
Bkassine	182	423	-	-	397	158	114	201
Antalya-Kumluca-Kalm	174	405	95	194	379	150	113	202
Yalova-Dumanlidag	185	455	78	196	392	-	132	-
Canakkale-Eceabat	190	456	101	199	393	142	124	204
Mugla-Yatagan-Katran	159	432	97	163	383	139	118	207
St. Raphaël (Var)	175	418	114	200	379	-	123	207
Villeneuve (Hérau)	174	456	101	196	396	149	128	210
Saintes-Maries (B.du)	192	459	125	188	391	158	122	196
St. Aygulf (Var)	186	438	112	210	381	-	137	211
Brignoles- Le Val	204	470	121	200	415	163	134	217
Alcaccer do Sal	183	434	107	179	-	-	-	-
Serra do Minho	186	480	122	193	-	-	-	-
Cogeces de Iscar (VA)	197	482	126	212	-	-	-	-
Valorio, fondo (ZA)	202	478	126	200	-	-	-	-
Valorio, ladera (ZA)	216	502	138	201	-	-	-	-
Toro (ZA)	211	481	150	207	-	-	-	-
Vega Sicilia (VA)	211	467	127	200	-	-	-	-
Tordesillas (VA)	192	473	128	200	-	-	-	-
Portillo (VA)	179	450	120	201	-	-	-	-
Íscar (VA)	200	461	127	214	-	-	-	-
Cadalso (M)	205	482	129	195	-	-	-	-
Budía (GU)	172	473	-	-	-	-	-	-
Biar (A)	193	468	107	224	-	-	-	-
Tarazona de la Mancha	193	484	112	202	-	-	-	-
Parafrugell (GI)	195	470	127	200	-	-	-	-
Garrovillas (CC)	170	435	112	185	-	-	-	-
Doñana (CA)	173	416	111	197	-	-	-	-
Las Lomas (CA)	190	442	97	209	-	-	-	-
Cartaya (H)	192	420	95	192	-	-	-	-
Adj. minimum mean height	159	405	78	163	379	139	113	196
Adj. minimum mean height	228	502	150	224	420	163	153	217
Observed minimum height	27	110	25	115	175	26	40	-
Observed maximum height	352	660	310	520	720	360	210	-
Site Mean	190	456	114	198	394	151	131	206

The residual analysis of a General Linear Model without spatial adjustment for each test site showed that the original experimental designs in blocks were not able to capture this variation at smaller scales. After applying instead of the pre-blocking nearest-neighbours adjustments (NNA), the spatial patterns accounted for 73-78% of the overall variance at each site, and the significance of the geographic genetic variation increased considerably (not so much due to changes in the adjusted provenance means than due to the huge reduction of the residual noise, thus narrowing confidence intervals), showing a common trend between sites.

At the Spanish test sites, the conclusions coincide with previous ones from 2003 (Mutke *et al.*, 2010): most provenances from inner Spain and one from France and the Italian one achieved the highest growths, whereas especially warmer, coastal provenances from southern Turkey, southern Spain and the French Côte d'Azur grew less (Table 2).

Applying a Principal Component Analysis of the adjusted mean tree heights for 29 provenances only from the four Spanish test sites, only the first component *CP1Sp* was retained. It accounted for 65% of the observed variance and correlated significantly with the mean annual temperature (MAT) in origin as climate proxy for each provenance (coefficient of correlation $r = -0.68$). The colder provenances did not only grow more, but showed also an earlier spring flush and higher tendency to form Lammas shoots (polycyclism) (Mutke *et al.*, 2010).

Analysing only the eight provenances present at all seven studied test sites in both countries, the first principal component accounted for 58% of the variance (55% when including the provenance means at Tunisian sites). This first principal component had a narrow correlation (0.98) with the values of *CP1Sp* from Spain and an negative correlation with mean annual temperature (MAT) in origin even stronger than the former variate ($r = -0.82$). Actually, the correlations of the adjusted provenance mean heights at each test with MAT ranged from -0.25 (La Gaillarde) to -0.65 (Tordesillas), -0.41 for the mean heights adjusted in Tunisia.

IV – Conclusions

In spite of the reduced molecular base of genetic variation in neutral molecular markers described for *Pinus pinea* (Vendramin *et al.*, 2008), the study revealed adaptative differences among populations, with the same trend observed already in a former analysis of the Spanish test sites, with lower growth in accessions from warmer origins (Mutke *et al.*, 2010). But the phenotypic plasticity of the species, especially sensitivity to soil variation, produced strong spatial autocorrelations of the response variables that masked greatly this variation, requiring nearest-neighbours adjustments for capturing the spatial pattern at each site.

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Characterization of *Pinus pinea* L. and *P. halepensis* Mill. provenances from Spain and Tunisia related to their rootstock use

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Abstract. Experiences carried out on the last years for exploring the possibilities of stone pine as an orchard crop have rarely been focused in the study of rootstocks and their effect on phenology and cone production. Thus, on the framework of PCI project, rootstock behaviour trials of different *P. pinea* and *P. halepensis* provenances have been planned, including an early characterization under controlled conditions, complemented on the following years with a field trial network where these plant materials will be grafted and established on different edaphic and climatic conditions in Spain and Tunisia. In 2010, seedlings of 8 Spanish and Tunisian Stone pine ('Remel', 'Mhibes', 'Aiguafreda', 'Caldes de Malavella') and Aleppo pine ('Kef', 'Thibar', 'Sallent', 'Palau-Sator') provenances were submitted to 3 water stress levels and two substrate typologies in a split-split-plot experimental design carried out at IRTA's station Torre Marimon (Caldes de Montbui, Spain). Data recorded included growth and biomass, physiologic parameters (RWC, CT, $\delta^{13}\text{C}$) and ontogenical information. Higher intra-specific variability and a more clear response to the different treatments have been observed in *P. halepensis*. Tunisian *P. halepensis* provenances showed higher biomass allocation in the more stressful conditions (particularly 'Thibar'), but displayed significant reductions on allocated biomass in sandy substrate (particularly 'Kef'); they also showed lower RWC values and a faster ontogeny (vegetative phase change).

Keywords. Aleppo pine – Stone pine – Provenance trial – Water stress – Substrate.

Caractérisation de différentes provenances de *Pinus pinea* et *P. halepensis* d'Espagne et de Tunisie par rapport à leur emploi comme porte-greffes

Résumé. Les expériences réalisées ces dernières années explorant les possibilités du pin pignon comme culture fruitière ont rarement été orientées à l'étude des porte-greffes et de leur influence sur la phénologie et la production de cônes. Ainsi, dans le cadre d'un projet PCI, des essais de caractérisation comme porte-greffes de différentes provenances de *P. pinea* et *P. halepensis* ont été planifiés, comprenant une évaluation précoce en conditions contrôlées, qui sera complétée ultérieurement par un réseau d'essais de terrain où ces matériels végétaux seront greffés et établis sous différentes conditions édapho-climatiques en Espagne et en Tunisie. En 2010, des plants de 8 provenances d'Espagne et de Tunisie de Pin pignon ('Remel', 'Mhibes', 'Aiguafreda', 'Caldes de Malavella') et de Pin d'Alep ('Kef', 'Thibar', 'Sallent', 'Palau-Sator') furent soumis à trois niveaux de stress hydrique et à deux typologies de substrats dans un dispositif expérimental en split-split-plot conduit à l'IRTA Torre Marimon (Caldes de Montbui, Espagne). Les données collectées incluent croissance et biomasse, paramètres physiologiques (RWC, CT, $\delta^{13}\text{C}$) et information concernant la différenciation ontologique. Une plus grande variabilité intra-spécifique et une plus claire réponse aux différents traitements ont été observées chez *P. halepensis*. Les provenances tunisiennes de *P. halepensis* (surtout 'Thibar') montrèrent une plus grande fixation de biomasse dans les conditions les plus stressantes, tandis que la biomasse fixée en substrat sableux fut significativement réduite (surtout pour 'Kef'); elles montrèrent également des valeurs plus faibles de RWC et un changement plus rapide de phase végétative.

Mots-clés. Pin d'Alep – Pin pignon – Essai de provenance – Stress hydrique – Substrat.

I – Introduction

Experiences carried out for exploring the possibilities of stone pine as an orchard crop have been mainly focused on the evaluation of their productive potential, in elucidating the relevance of environmental factors and in the selection of the best productive genotypes (Catalán Bachiller 1990; Mutke Regneri *et al.*, 2003; Mutke *et al.*, 2005a; Mutke *et al.*, 2005b; Mutke *et al.*, 2007). However, there is very little information (Climent *et al.*, 1997) concerning which is the suitable plant material to use as rootstock in each environment and how it affects the phenology and fruitfulness in grafted plantations.

On the framework of a PCI project, behaviour trials of different *P. pinea* and *P. halepensis* provenances under controlled conditions have been undertaken to make an early characterisation for its use as rootstocks. The main objective of the trial carried out at Torre Marimon IRTA's station has been to study the behaviour of different *P. pinea* and *P. halepensis* provenances against water stress. On the following years, this nursery characterization will be complemented with a field trial network on different edaphic and climatic conditions in Spain and Tunisia.

II – Material and methods

1. Plant material and experimental design

For the conduct of this experience a total of 8 provenances have been chosen in different ecological environments of Tunisia and Spain, trying to cover a wide range of ecological conditions (Fig. 1, Tables 1 to 3). Four of them were Aleppo pine provenances ('Kef', 'Thibar', 'Sallent' and 'Palau-Sator') and the other four stone pines ('Remel', 'Mhibes', 'Aiguafreda' and 'Caldes de Malavella'). 'Palau-Sator' and 'Caldes de Malavella' are coastal provenances, whereas 'Sallent' and 'Aiguafreda' are more continental; Tunisian stone pine provenances have a sub-humid climate, whereas Aleppo pine provenances come from semi-arid regions.

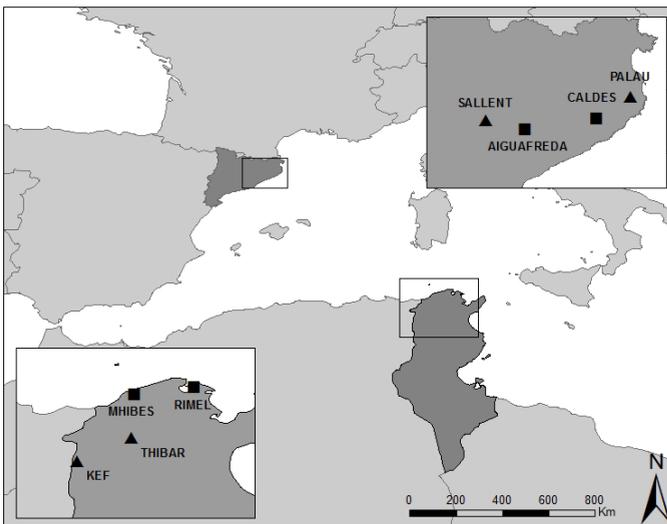


Fig. 1. Location of the *Pinus pinea* (■) and *P. halepensis* (▲) provenances.

Table 1. Geographical origin of provenances used in the trial

Provenance	Species	Origin	Long	Lat
Palau-Sator	<i>P. halepensis</i>	Spain	3° 6'	41° 58'
Sallent	<i>P. halepensis</i>	Spain	1° 56'	41° 49'
Kef	<i>P. halepensis</i>	Tunisia	8°23'	36°12'
Thibar	<i>P. halepensis</i>	Tunisia	9° 7'	36°31'
Caldes Malavella	<i>P. pinea</i>	Spain	2° 49'	41° 50'
Aiguafreda	<i>P. pinea</i>	Spain	2° 15'	41° 46'
Mhibes	<i>P. pinea</i>	Tunisia	9° 9'	37° 7'
Rimel	<i>P. pinea</i>	Tunisia	9°58'	37°13'

Table 2. Ecological characterization of Spanish provenances used in the trial

Provenance	Altitude	Provenance region	Climate subtype	Soil [†]	Texture	pH
Palau-Sator	50-100	High Catalonia	Subnemoral Mediterranean	LVh	Loam	8.2
Sallent	400-500	Inner Catalonia	Nemoral subesteparian	CMc	Clay loam	8.5
Caldes de Malavella	120-135	Coastal Catalonia	Nemoromediterranean submediterranean	CMd/u	Sand loam	6
Aiguafreda	465-630	Inner Catalonia	Nemoral subesteparian	CMd	Loam	8.3

[†]LVh: Haplic Luvisol; CMc: Calcaric Cambisol; CMd/u: Dystric/Humic Cambisol; CMd: Dystric Cambisol.

Table 3. Climatic characterization of Tunisian provenances used in the trial

Provenance	Location	P	T	t _m	T _M	Bioclimate
Kef	North-West Tunisia	446	16.3	3.3	34	Semi-arid Mediterranean, temperate winter
Thibar	North-West Tunisia	612	17.9	5.7	35.3	Semi-arid Mediterranean, mild winter
Mhibes	North Tunisia	829	17.4	-	-	Sub-humide Mediterranean, mild-warm winter
Rimel	North Tunisia	610	18.1	7.6	31.1	Sub-humide Mediterranean, warm winter

P: annual rainfall; T: mean annual temperature, t_m: average of minimum temperatures of coldest month; T_M: average of minimum temperatures of warmest month.

Seeds were sown in April 2009 into Forest Pot-200 air-slit forest trays. In November 2009 seedlings were transplanted into Coneplast C-20R containers (2.5 l) containing two sorts of mixture: half of the total plants produced were transplanted into the Substrate 1 (S1), constituted by peat and vermiculite (2:1); the other half were transplanted into Substrate 2 (S2), constituted by peat, vermiculite and sand (1:1:1). Plants were maintained in a forest nursery until spring 2010; in June 2010 plants entered into greenhouse and the application of the different irrigation regimes started in July 2010. This experience lasted until November 2010.

Three water stress levels were applied, corresponding to minimum soil water contents of 20%, 10% and 5%, (cm^3 water/ cm^3 substrate). Water supply was always of 333 ml/plant, but the frequency of application varied between treatments (Table 4). The substrate water content was monitored with a set of TDR probes (time-domain reflectometry). This information was useful to modify the frequency of irrigation in the different periods of the experiment. The experimental design was a split-split-plot with 3 replications and 20 plants per experimental unit (total size 2520 plants), with irrigation regime, substrate and provenance as factors.

Table 4. Irrigation treatments scheduling

Irrigation regime	7 Jul – 29 Jul	30 Jul – 1 Sep	2 Sep – 14 Nov	SWC [†]
R1	333 ml / 2 days	333 ml / 3 days	333 ml / 3 days	20%
R2	333 ml / 3 days	333 ml / 5 days	333 ml / 6 days	10%
R3	333 ml / 4 days	333 ml / 7 days	333 ml / 9 days	5%

[†]Minimum Soil Water Content, prior to re-watering.

2. Measurements

Data were recorded at three different moments, at the beginning, middle and end of the trial (final June, final August and November). In each sampling date, plant height, plant diameter and biomass determinations of each portion, below and aboveground (root, stem, needles) were registered. These data were used to define some relative variables (allocated biomass of each fraction and root/shoot ratio). Moreover, some physiologic parameters, relative water content (RWC) and cuticular transpiration (CT) were recorded. Water use efficiency (WUE_i) was estimated from the isotopic carbon content ($\delta^{13}\text{C}$) analysis of some control plants not subjected to water stress. Finally, ontogenetic information (adult needle proportion and winter buds setting) was also noted down.

III – Results

1. Differences between species

At species level, very significant differences were observed in final biomass, total allocated biomass and allocated aerial biomass, with higher values in *P. pinea* in all the irrigation regimes and for the two types of substrate; root/shoot ratio at the end of the trial and root/shoot increase from the middle to the end of the trial were significantly higher in *P. halepensis*, in all the irrigation regimes and for the two types of substrate (Table 5).

Table 5. ANOVA for different biomass variables

Species	B _f (g)	R/S _f	Δ R/S	Δ B (g)	Δ B _{root} (g)	Δ B _{aer} (g)
	***	***	***	***	N.S.	***
<i>P. halepensis</i>	12.78 b	0.58 a	0.17 a	8.33 b	2.63	5.71 b
<i>P. pinea</i>	19.21 a	0.39 b	0.09 b	10.43 a	2.51	7.92 a

B_f: Biomass at the end of the trial; R/S_f: root/shoot at the end of the trial; Δ R/S: root/shoot increase; Δ B: Total allocated biomass; Δ B_{root}: Total allocated root biomass; Δ B_{aer}: Total allocated aerial biomass. Duncan ($\alpha=0.05$); N.S.: non-significant; ***: significant $P<0.001\%$.

Concerning the physiological variables, very significant differences between species were observed in cumulated cuticular transpiration rate and relative water content, with higher values

in *P. pinea* in all the irrigation regimes and for the two types of substrate; the proportion of secondary needles was always significantly higher in *P. halepensis*; no significant differences at species level were found for the isotopic carbon content (Table 6).

Table 6. ANOVA for different physiological variables

Species	CCT _m (slope)	RWC _m (%)	Sn (% dry weight)	δ ¹³ C (‰)
	***	***	***	N.S.
<i>P. halepensis</i>	0.044 b	88.09 b	10.25 a	27.71
<i>P. pinea</i>	0.049 a	91.86 a	0.87 b	27.93

CCT_m: Cumulated cuticular transpiration rate in the middle of the trial; RWC_m: needles relative water content in the middle of the trial; Sn: Proportion of secondary needles at the end of the trial; δ¹³C: isotopic carbon content. Duncan ($\alpha=0.05$); N.S.: non-significant; ***: significant $P<0.001\%$.

2. Differences within species

A. *Pinus halepensis*

Although there are no significant differences, Tunisian *P. halepensis* provenances, and particularly 'Thibar', showed a slightly higher biomass allocation in the more stressful conditions (Fig. 2a). Tunisian *P. halepensis* provenances, and particularly 'Kef', displayed significant reductions on allocated biomass with the sandy substrate; 'Palau-Sator' and 'Sallent' are much less affected by the type of substrate (Fig. 2b).

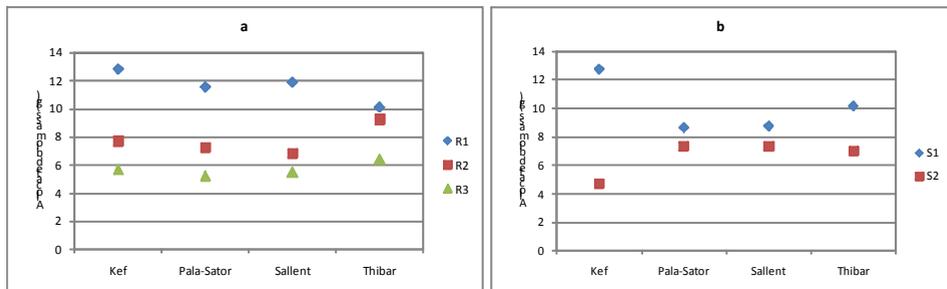


Fig. 2. Allocated biomass of *P. halepensis* provenances: response to water stress levels (a) and to the type of substrate (b).

'Palau-Sator' shows the highest root to shoot ratios and 'Kef' the lowest ones (Fig. 3, Table 7); however, 'Sallent' and 'Thibar' undergo a more clear response to the water stress treatments, with a significant increase of shoot to root ratios in the more stressful conditions (Fig. 3).

Tunisian Aleppo pine provenances had the lowest relative water content values, in both favourable and stressful scenarios (Table 7). Concerning isotopic carbon content, we found highly significant differences within *P. halepensis* provenances: 'Palau-Sator' and 'Sallent' are placed on the highest and the lowest WUE_i position, respectively; Tunisian provenances ranged in intermediate position (Table 7).

Tunisian Aleppo pine provenances (and particularly 'Kef') showed a faster ontogeny (vegetative phase change) represented by a higher proportion of secondary needles (Table 7) and a higher

frequency of budset (data not shown). Budset and occurrence of secondary needles was always lesser in sandy substrate (Fig. 4).

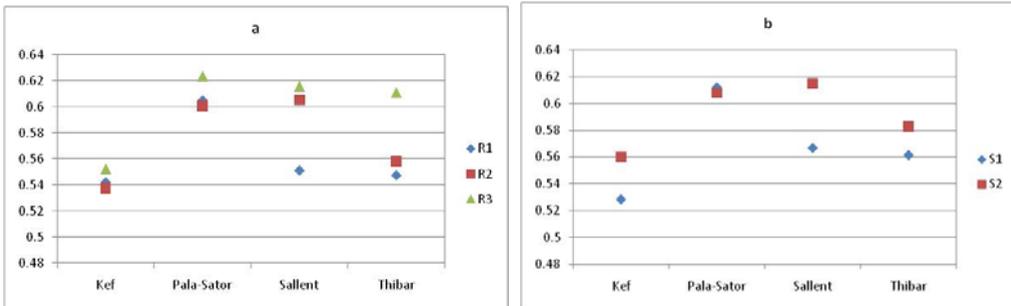


Fig. 3. Root to shoot ratio of *P. halepensis* provenances: response to water stress levels (a) and to the type of substrate (b).

Table 7. ANOVA between *P. halepensis* provenances for different variables

Provenance	Root/shoot	RWC _m (%)	Sn (% dry weight)	δ ¹³ C (‰)
	*	***	***	***
Palau-Sator	0.61 a	90.2 a	3.0 c	-27.24 a
Sallent	0.59 ab	89.2 a	1.7 c	-28.17 c
Thibar	0.57 bc	86.6 b	14.9 b	-27.48 b
Kef	0.54 c	86.4 b	20.8 a	-27.95 c

RWC_m: needles relative water content in the middle of the trial; Sn: Proportion of secondary needles at the end of the trial; δ¹³C: isotopic carbon content. Duncan (α=0.05); N.S.: non-significant; *: significant P<0.05%; ***: significant P<0.001%.

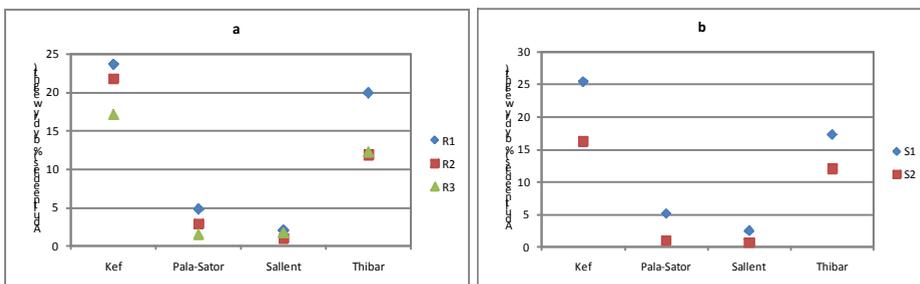


Fig. 4. Percentage (by dry weight) of secondary needles in *P. halepensis* provenances: response to water stress levels (a) and to the type of substrate (b).

B. *Pinus pinea*

Concerning water stress treatments, there are no significant differences in allocated biomass between *P. pinea* provenances (Fig. 5a). On the other hand, the type of substrate entails significant differences between provenances: sandy substrate has a negative effect in Tunisian *P. pinea* provenance, whereas 'Caldes de Malavella' seems to be indifferent to the type of

substrate (Fig. 5b). Tunisian *P. pinea* has the higher root to shoot ratio, even in the more stressful conditions (Table 8).

There are no significant differences between *P. pinea* provenances in relative water content and isotopic carbon content (Table 8). Occurrence of secondary needles was very low in *P. pinea* provenances (Table 8); we also observed lesser budset in Tunisian stone pines (data not shown).

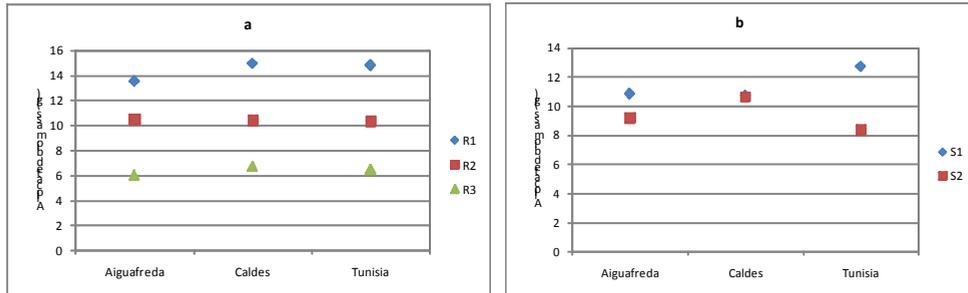


Fig. 5. Allocated biomass of *P. pinea* provenances: response to water stress levels (a) and to the type of substrate (b).

Table 8. ANOVA between *P. pinea* provenances for different variables

Provenance	Root/shoot	Sn (% dry weight)	$\delta^{13}\text{C}$
	***	N.S.	N.S.
Tunisia	0.41 [†] a	1.01 [†]	-27.89 ^{††}
Aiguafreda	0.37 b	0.88	-27.94
Caldes de Malavella	0.40 a	0.72	-27.95

Sn: Proportion of secondary needles at the end of the trial; $\delta^{13}\text{C}$: isotopic carbon content. Duncan ($\alpha=0,05$); N.S.: non-significant; ***: significant $P<0,001\%$

[†]'Tunisia' provenance is constituted by 'Rimel' and 'Mhibes' seedlings.

^{††}The value of isotopic carbon content corresponds only to Rimel provenance.

IV – Conclusions

In general, higher intra-specific variability and a more clear response to the water stress treatments and to the different substrates have been observed in *P. halepensis*, whereas *P. pinea* shows low variability in most of the variables analyzed. Stone and Aleppo pine Tunisian provenances are much more affected by the type of substrate than the Spanish ones.

The aim of this study was to make an early characterization of different plant materials in front of water stress. The next step will be to graft these plant materials and to establish a network of experimental plots in different ecological conditions, in Spain and in Tunisia, in order to study its field behaviour and the influence of different rootstocks in growth, phenology and cone production of grafted materials.

Acknowledgments

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Cloning stone pine (*Pinus pinea* L.) by somatic embryogenesis

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Abstract. Somatic embryogenesis (SE) is the biotechnology of propagation that enables to put multi-varietal forestry (MVF) into practice. MVF involves the use of genetically tested varieties in plantation forestry, balancing genetic gain with diversity. Embryogenic lines can be cryopreserved in liquid nitrogen while corresponding trees are tested in the field, and those showing genetic superiority can be recovered for mass production of elite plants. The SE process in pines involves the initial outgrowth of embryo-suspensor masses (ESM) from immature zygotic embryos, the establishment of embryogenic cultures capable of further differentiation into somatic embryos, and their germination as somatic seedlings. We reported plant regeneration by SE in stone pine, and further research is in progress to improve the efficiency of the several steps. Induction was obtained at very low frequency (0.4%), and the best embryogenic line and maturation condition yielded 220 mature embryos per gram of ESM. Germination was achieved at 70%, and 35 % of somatic embryos were converted into plants. Suspension cultures offer advantages over semisolid cultures for mass propagation. The establishment of ESMs of stone pine in liquid medium is reported, and the recovery of mature embryos from tissues grown in liquid culture is also described. Current main bottlenecks of SE for implementing MFV in stone pine are the low induction rates and the cessation of growth of converted somatic seedlings.

Keywords. Biotechnology – High yielding varieties – *In vitro* culture – Tree breeding – Vegetative propagation.

Clonage de pin pignon (*Pinus pinea* L.) par embryogenèse somatique

Résumé. L'embryogenèse somatique (ES) est la biotechnologie de propagation qui permet de mettre la culture forestière multi-variétale (FMV) en pratique. La FMV implique l'utilisation de variétés génétiquement testées en plantation en exploitant au mieux l'équilibre entre le gain génétique et la diversité. Les lignées embryogènes peuvent être cryoconservées dans l'azote liquide, tandis que les arbres correspondants sont testés sur le terrain. Ceux qui montrent une supériorité génétique sont sélectionnés pour la production en masse de plants d'élite. Le processus d'ES du pin implique la croissance initiale des masses d'embryons et de suspenseurs (ESM) à partir d'embryons zygotiques immatures, l'établissement de cultures embryogènes capables de différenciation en embryons somatiques matures, et la germination de ceux-ci. Nous présentons la régénération des plantes de pin pignon par ES et les recherches qui sont en cours pour améliorer l'efficacité des différentes étapes. L'induction a été obtenue à très basse fréquence (0,4%). La meilleure lignée et le meilleur traitement de maturation ont fourni 220 embryons matures par gramme d'ESM. La germination a atteint 70%, et 35% des embryons germés se sont convertis en plantes. Les cultures en suspension offrent des avantages par rapport aux cultures sur milieux semi-solides pour la propagation en masse. L'établissement d'ESM en milieu liquide et l'obtention d'embryons matures de pin pignon à partir de ces cultures sont décrits. Les principales étapes limitantes de l'ES pour mettre actuellement en application la FMV pour le pin pignon sont les faibles taux d'induction et l'arrêt de croissance des plantules somatiques.

Mots-clés. Amélioration des arbres – Biotechnologie – Culture *in vitro* – Multiplication végétative – Variétés à rendement élevé.

I – Introduction

1. Clonal forestry and multi-varietal forestry

The demand of forest products is constantly growing. The forecasts for timber requirements at the half of this century are six billions of cubic meters (Sutton, 2000). Both the recycling of paper and the establishment of forest plantations that can respond to the growing needs of pulp, timber and other products, are considered the best defence of native forests threatened by deforestation (Sutton, 1999). Forest plantations with intensive production may alleviate the great pressure on natural ecosystems. In Brazil, for example, high-yield *Eucalyptus* plantations, which account for only 2% of the country's entire crop, are responsible for 60% of their nation's production. In Chile and New Zealand plantations of radiata pine covering about one fifth of the total area are responsible of more than 90% of the total production (Sedjo, 1999). Actually, at present forest plantations constitute only five per cent of global forest cover but already supply up to 35 per cent of global round wood (Carle *et al.*, 2005).

Cultural and genetic improvement practises have to be applied to increase yield in intensively managed forest plantations. Plant propagation is one of the major issues in tree breeding and genetic resource conservation. The more powerful tool that has been used in genetic improvement of many woody crops such as wines, fruit trees and olive trees is vegetative propagation. Cloning plays also an important role in breeding forests species (Zobel and Talbert, 1984). In fact, species of the genus *Populus*, *Salix*, *Cryptomeria* and *Eucalyptus* that are amenable of vegetative propagation are intensively managed using this form of propagation.

However many forest species are recalcitrant to vegetative propagation. Currently, plant biotechnology may solve problems linked to the obtaining vegetative propagules at very high amount and reduced cost. But forest biotechnology must be coupled with classical breeding schemes in order to obtain their full advantages. The strategy of multi-varietal forestry (MVF) (Park, 2004) which is based on the former clonal forestry (Park, 2002) and is defined as the use of genetically tested tree varieties in plantation forestry, balancing genetic gain with diversity, begins to be implemented due to the development of embryogenic systems. Plant regeneration by somatic embryogenesis (SE), combined with cryopreservation, offers an opportunity to develop highly valuable clonal varieties. Embryogenic lines can be cryopreserved in liquid nitrogen maintaining their regeneration potential while corresponding trees are tested in the field. Clonal lines that have shown genetic superiority can be recovered after thawing for mass production of elite plants. Thus clonal propagation by SE can quickly capture the benefits of breeding and can be implemented in a flexible way, so it is expected to play an important role in increasing productivity, sustainability and uniformity of many forest plantations.

2. The embryogenic system in conifers and its commercial application

The embryogenic system as a way of plant regeneration is the basis of several applied biotechnologies (clonal propagation, cryopreservation, genetic transformation) that are used for the conservation and improvement of genetic resources. The implementation of MVF for *Pinus* species requires a SE system with high initiation and plant conversion rates, maintaining the genetic integrity; hence the development of optimized protocols is needed to bring this biotechnology to its full potential. After the first reports in conifers (*Picea abies*, *Larix decidua*) in 1985, SE has been achieved in many pine species as *Pinus pinaster*, *P. radiata*, *P. strobus* and *P. taeda*. In this last species SE is currently being used at the operational practice by private companies. The SE process in pines involves the initial outgrowth and continuous growth of embryo-suspensor masses (ESM) from immature zygotic embryos, allowing the establishment of embryogenic cultures capable of further differentiation into somatic embryos. Then somatic embryos germinate giving somatic seedlings. Growth rates of ESM are usually fairly high ensuring a high multiplication potential. However maturation and quality of somatic embryos are limiting factor to conversion into plants.

The protocols for regeneration of conifer species by SE have been considerably improved in the last years (Klimaszewska *et al.*, 2007). These improvements have generated a strong commercial activity of production of clonal plants mainly in the species *Pinus taeda*, *Pinus radiata* and *Pseutsuga menziesii*. In fact private biotechnological companies are making alliances with classical forest corporations to apply the recent developments in SE. For instance the US company ArborGen [<http://www.arborgen.com/>], whose motto is *more wood, less land*, signed joining agreements with International Paper, MeadWestvaco, and Weyerhaeuser to produce and test improved loblolly pine varieties. The Canadian company Cellfor [www.cellfor.com] is producing and trading more than 27 million plants of loblolly pine per year (Park, 2007). Several other companies are using SE to establish clonal tests and starting commercialisation: BioForest and GenFor (Chile), Carter-Holt Harvey, Rubicon and Rayonier (New Zealand) and JD Irving (Canada) (Cyr and Klimaszewska, 2002). In Europe practical applications are less developed but recently a company has been created in Sweden, SweTree Technologies [<http://www.swetree.com/index.html>]. In collaboration with traditional forest companies are developing industrial methods to produce elite embryogenic lines of Norway spruce at large scale. They plan to have by 2012 a pilot production system which will have a capacity of producing millions of plants per year.

An attribute of increasing interest in the last years is the epigenetic control on the phenotype. It has been observed that the temperature during maternal reproduction affects adaptive traits: independent tests in several conifer tree species have shown that growth, bud phenology and frost hardiness of progenies are influenced by the climatic conditions during sexual reproduction, and the memory effects seem to endure for many years in the filial generation. Elevated temperature during female flowering revealed that the period from embryogenesis through seed maturation is the most likely sensitive period (Johnsen *et al.*, 2005). Epigenetic changes in gene expression that can be transmitted from one generation to the next are supposed to be behind this effect. This “epigenetic memory”, which is expressed as a long-lasting effect on height growth and bud phenology in the progenies, has also been evidenced using somatic plants produced by SE under different temperature regimes (Kvaalen and Johnsen, 2008). This aspect can be of great interest in the case of stone pine, a species that is claimed to have virtually no neutral genetic variation, but show enough phenotypic plasticity for spreading over a very large and environmentally diverse area (Mutke *et al.*, 2010). Somatic plants can be very useful to study this phenomenon.

II – The embryogenic system in stone pine

Taking into account the economic importance of the pine nut production, breeding programs of stone pine have begun, aiming at improved cone production (Mutke *et al.*, 2005). These programs are based on the selection of elite trees for grafting and planting in orchards designed for the production of its edible megagametophyte (Mutke *et al.*, 2007). Therefore the management of stone pine in plantation forestry for fruit production might require the development of rootstock varieties adapted to different environmental conditions.

Clonal rootstocks are widely used in the cultivation of many woody crops (Castle, 1995; Webster, A.D. 1997; Kester *et al.*, 2002). They can exert considerable effects over the performance of the scion and, consequently, over the tree growth, flowering and fruit size. In addition clonal rootstocks provide a uniform orchard that facilitates management. In forest species marked rootstock effects on growth and reproduction have been observed, and even some crown characters and nutrient content of scions can be manipulated by using specific rootstocks (Jayawickrama *et al.*, 1991). In Norway spruce clonal rootstocks reliably increased the number of flowering grafts. Hence the development of early, abundant and continuous flower-stimulating rootstocks was envisaged (Melchior, 1987). Consequently the development of vegetative propagation techniques for *Pinus pinea* should be desirable.

Attempts for cloning stone pine by micropropagation have been successfully carried out using

the organogenic pathway of regeneration (Alonso *et al.*, 2006; Cuesta *et al.*, 2008; Cortizo *et al.*, 2009). However the procedures are very laborious and rooting percentages are not high enough to consider this option for profitable propagation. At present, only SE is considered the option of choice for mass vegetative propagation of difficult-to-root forest species.

1. Initiation, proliferation and cryopreservation of embryogenic lines

We reported plant regeneration by SE in stone pine (Carneros *et al.*, 2009). Embryogenic lines were obtained from developing zygotic embryos by culturing immature seeds without coats (Fig. 1A). Different induction media containing several concentrations of an auxin (2,4-dichlorophenoxyacetic acid) and a cytokinin (6-benzylaminopurine) were tested to induce SE. Overall it was obtained at a very low frequency (0.4%). However differences related to the year of collection and half-sib family were found, with some families doubling the general mean. Within a year, the most responsible collection date was at the beginning of July, when zygotic embryos were at the post-cleavage state of development. By this time, although the extrusion of tissue from the micropilar end of the megagametophytes occurred on average at almost 10% of the explanted seeds, only a tenth of these proliferations became initiated as embryogenic lines. Some families had *temporal windows of response* broader than others. Also they showed different initiation frequencies, some of them reaching 2.5% of established embryogenic lines.

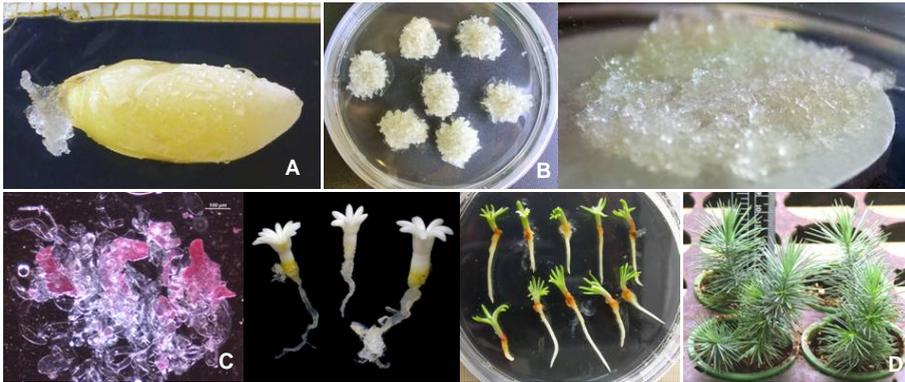


Fig. 1. Several steps of plant regeneration by somatic embryogenesis in *Pinus pinea*. **A:** Induction: outgrowth of embryogenic tissue from megagametophyte; **B:** Proliferation: growth of embryo suspensor masses on semisolid medium, as clumps (left) or dispersed on filter paper (right); **C:** Differentiation, maturation and germination of somatic embryos (from left to right); **D:** somatic seedling of stone pine in forest containers.

Proliferation of embryogenic lines takes place in conifers by cleavage polyembryony that sets a recurrent process of multiplication, forming masses of embryonal heads and suspensor cells. As the growth of these ESM is usually very active, this process is very productive. We tested with stone pine two procedures for the proliferation of this embryogenic tissue: growing as clumps or dispersed on filter paper (Fig. 1B). The second procedure was much more productive (Carneros *et al.*, 2009). Overall fresh weight of tissue growing as clumps was multiplied by 30 after three biweekly subcultures while it was multiplied by 70 when the dispersal procedure was used. In some genotypes this difference was even higher, reaching more than 10-fold.

A cryopreservation protocol for embryogenic lines of stone pine was developed (Carneros, 2009), that allows their long term conservation without loss of vigour and regeneration potential. Cryoprotective agents such as sorbitol and DMSO showed some toxicity that reverted after several subcultures. A pretreatment with these substances followed by a slow-freezing step

previous to immersion in liquid nitrogen were used. All cryopreserved lines recovered growth after thawing, but after a long lag phase. Mature embryos with germination ability could be obtained from two out of three tested lines.

2. Maturation of somatic embryos and conversion into plants

Differentiation and maturation of somatic embryos from ESM are needed to obtain clonal plants (Fig. 1C). First results showed a relatively low number of mature embryos, up to 36 embryos per gram of embryogenic tissue (Carneros *et al.*, 2009). This figure is in the range of the ones published in *Pinus nigra* (Salajova and Salaj, 2005) and *Pseudotsuga menziesii* (Gupta and Timmis, 2005). Then different factors were evaluated for improving embryo maturation and conversion. It was observed that the effect of ABA concentration was highly dependent on genotype. Medium with 121 or 161 μM ABA produced the highest number of mature embryos depending on the embryogenic line tested. The treatment that produced the most dramatic effect was the periodic subculture onto maturation medium, compared to continuous culture in this medium: repeated subculturing resulted in a 13-fold increase in mature embryos in two embryogenic lines. Tissue desiccation also enhanced maturation: a period of 2-4 hours under laminar flow conditions that caused a 7,5-17% relative water loss, increased the number of mature embryos up to almost double in one embryogenic line and 5-fold in another. The best embryogenic line and maturation condition yielded between 170 and 220 mature embryos per gram of ESM, which is in the range published in *Pinus taeda* (Pullman *et al.*, 2003) and *Pinus pinaster* (Lelu-Walter *et al.*, 2006). However, the inclusion of PCIB, an auxin inhibitor, in the maturation medium did not improve and even reduced the number of mature embryos as regards the control.

In average 70% of somatic germinated within four weeks. Conversion was enhanced (35%) when germinated embryos were transferred for further growth to vent culture boxes filled with SH medium (Carneros, 2009; Toribio *et al.*, 2011). Some somatic seedlings were transferred to forest containers (Fig. 1D).

3. Establishment of cultures in liquid media

Most protocols developed for cloning forest trees by SE are based on *in vitro* culture techniques using semisolid media. These protocols are useful to produce the reduced number of plants required to establish clonal tests necessary for validating selected varieties. However, for profitable mass production of improved planting stocks the development of culture in liquid medium is required. Liquid cultures offer technical advantages over semisolid cultures as a faster rate of growth based on the rapid uptake of nutrients by cells. In addition, the suspension culture system allows the study of physiological characteristic such as growth parameters, nutrient uptake and maturation capacity that are important for large scale production of somatic embryos in bioreactors. Furthermore, culture in liquid medium is required for the automation of cultures, lowering the high labour costs associated with tissue culture techniques. Culture in liquid medium and bioreactors has been developed for several conifer species (Gupta and Timmis, 2005)

We initiated suspension cultures from ESM of several embryogenic lines that are currently being maintained on semi-solid medium for more than five years retaining the embryogenic ability (Celestino *et al.*, 2011). Pieces of tissue were suspended in the same maintenance medium without gelling agent, and cultured in Erlenmeyer flasks on rotary shakers. The initial inoculum density was determinant for establishing the suspension, as was observed in other *Pinus* species (Salaj *et al.* 2007). Cultures evolved showing an initial lag phase followed by an exponential growth phase up to a maximum after which no further growth was detected, a typical pattern of suspension cell cultures (Azevedo *et al.*, 2008). Qualitative differences were observed in the growth of embryogenic masses depending on the degree of agitation. Higher orbiting speed caused more disaggregated and homogeneous proliferations (Fig. 2) that

produced more packed suspensions and reduced the settle cell volume recordings (Fig. 3). Embryo-suspensor masses showed more structured somatic embryos when were grown on solid medium than in liquid medium. However, mature embryos could be obtained from tissues grown in suspension cultures, though differentiation and maturation required plating the embryo-suspensor masses from liquid to semi-solid medium.

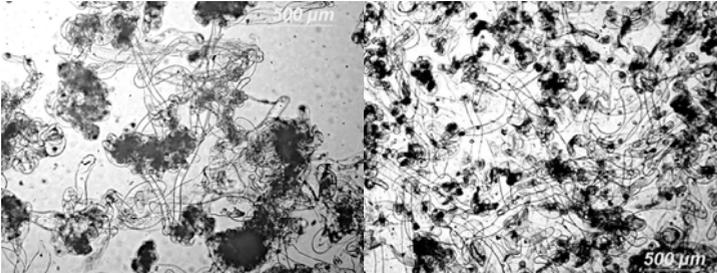


Fig. 2. Qualitative differences of embryogenic tissues grown at 50 rpm (left) and 100 rpm (right). Higher orbiting speed caused more disaggregated and homogeneous proliferations.

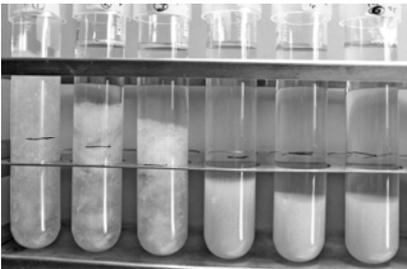


Fig. 3. Settle cell volumes recorded in embryogenic cultures growing at 50 rpm (left) and 100 rpm (right) that show much more packed suspensions.

III – Conclusions

Although methods for cloning stone pine by somatic embryogenesis have been defined, further research is needed to improve the efficiency of the several steps of the process. At present the main bottleneck is the very low induction rate. Genetic improvement programs needs to work with as much as possible genetic variability to avoid the risk of genetic erosion. Overall, one of the main drawbacks of the SE technology in forest species is the low frequency of initiation, which reduces the number of genotypes captured for breeding, and the recalcitrance of some high-value crosses to initiate embryogenic tissue. This might be solved on one hand by improving the present methods. Frequencies of induction described in the first studies in other species were quite low, contrasting with the frequencies reported several years later using optimised protocols. For instance, in Douglas-fir first data were in the range of only 1-2% proliferating ESMs after six months in culture (Durzan and Gupta, 1987). Nowadays with improved protocols initiation frequencies and establishment of embryogenic lines in Douglas-fir are in the range of 40-57% (Pullman *et al.*, 2009). A relevant characteristic of the induction

process in stone pine is that extrusion rates are much higher than the initiation ones. Therefore a technique such as liquid overlay could be useful for enhancing initiation of embryogenic lines (Pullman and Skryabina, 2007). On the other hand, it is known that the different phases of plant regeneration by SE are genetically controlled (Park *et al.*, 1993; Park *et al.*, 1994). Thus this fact opens the possibility of breeding for higher induction rates, looking for families with the highest induction frequencies and using the mother trees as female parents in controlled crosses (MacKay *et al.*, 2006).

Germination of mature embryos and conversion into somatic seedlings are limiting steps of SE in some species. In the case of stone pine, reasonable high percentages of germination (growth of both root and plumule) are obtained. However, after germination a high percentage of these plants stop growing. This current bottleneck reduces the efficiency of the SE process. Some studies on the effect of light quality on these steps have shown that subsequent seedling growth was promoted by red light (Merkle *et al.*, 2005). This could be useful to improve current methods in the stone pine.

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Chemical profiling of Portuguese *Pinus pinea* L. nuts and comparative analysis with *Pinus koraiensis* Sieb. & Zucc. commercial kernels

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Abstract. Being an endemic species to the Mediterranean Basin, stone pine (*Pinus pinea* L.) obtains an extreme economical relevance in Spain, Portugal, Italy, Tunisia and Turkey, where pine nuts are traditionally marketed and consumed. Based on the analysis of 27 different Portuguese populations, pine nuts were characterized by high contents of fat (47.7 g per 100 g dry matter DM), protein (33.9 g per 100 g DM) and phosphorus (1130 mg per 100 g DM) and low contents of moisture (5.9 g per 100 g DM) and starch (3.5 g per 100 g DM). They were also found to be a good source of zinc, iron and manganese. A comparative analysis with *P. koraiensis* nuts showed the latter to have lower protein (14.1 g per 100 g DM) vitamin and mineral contents, and higher fat (68.1 g per 100 g DM) and pinolenic acid contents (14.5% vs 0.3% w/w TFA). Protein and pinolenic acid contents could be considered as good parameters to distinguish the edible kernels of these two species.

Keywords. *Pinus pinea* – *Pinus koraiensis* – Proximate analysis – Fatty acids – Mineral analysis.

Profil chimique du pin pignon portugais *Pinus pinea* L. et analyse comparée avec *Pinus koraiensis* Sieb. & Zucc.

Résumé. Le pin pignon (*Pinus pinea* L), espèce endémique au bassin méditerranéen, a un poids économique considérable dans quelques régions d'Espagne, du Portugal, d'Italie, de la Tunisie et de la Turquie où, traditionnellement, le pignon de pin est commercialisé et consommé. En se basant sur l'analyse de 27 populations portugaises différentes, le pignon de pin a été caractérisé par des teneurs élevées en graisse (47.7 g pour 100 g de matière sèche MS), en protéines (33.8 g pour 100 g MS) et en phosphore (1130 mg pour 100 g MS) et de faibles teneurs en humidité (5.9 g pour 100 g de MS) et en amidon (3.5 g pour 100g MS). De plus il s'est révélé être une bonne source de zinc, de fer et de manganèse. Une analyse comparative avec le pignon de *P. koraiensis* a montré que celui-ci a des teneurs en protéines (14.1 g MS pour 100 g) et en vitamines et minéraux plus faibles. Par contre, les teneurs en acides gras (68.1 g pour 100 g MS) et en acide pinolénique (14,5% vs 0,3% p/p du total en acides gras) sont plus élevées. Les teneurs en protéines et en acide pinolénique pourraient être considérées comme des paramètres à retenir pour différencier le pignon comestible de ces deux espèces.

Mots-clés. *Pinus pinea* – *Pinus koraiensis* – Analyse immédiate – Acides gras – Analyse minérale.

I – Introduction

The Portuguese area of stone pine forest is estimated in 130,000 hectares (IFN5, 2010). Due to the species plasticity, *P. pinea* can be found throughout the country. Among the seven provenance regions delineated in the Portuguese territory (Cardoso and Lobo, 2001), provenance region 5 (South of Tagus) gathers about 62% of the total area due to the particular

ecological conditions. Specifically the district of Setúbal, region of Alcácer do Sal, stands out as the most important pine-nut production area due to both yield and quality, concentrating more than 50% of the national production and rendering the region economy highly dependent on pine nut exploitation.

The seeds of *P. pinea* are ancient components of the Mediterranean diet, included as ingredients in confectionery and cooking for its exquisite flavor. Recently, there has been a growing worldwide market for pine nuts. In consequence, a strong competition has been generated by pine nuts of other species, mostly *P. koraiensis* Sieb. & Zucc. from China, which reach the local markets at lower prices and are undistinguishable to the untrained eye of consumers, although they have a different flavor, shape and size.

Considering that the origin is an important issue for producer and consumer protection, this study aimed to comparatively analyse the chemical profiles of *P. pinea* nuts and Chinese pine nuts (*P. koraiensis*) and specifically find parameters to distinguish them.

II – Material and methods

Local sampling was performed on 27 Portuguese populations from three *Pinus pinea* Provenance Regions and from four external locations. A commercial sample of Chinese pine nuts (*Pinus koraiensis* Sieb. & Zucc.) was also included for comparative analysis (Fig.1A, B). The following chemical analyses were performed: proximate analysis (moisture, crude protein, crude fat, and starch); vitamin analysis (thiamine and riboflavin); fatty acid analysis saturated fatty acids (SFA) palmitic, stearic and arachidic; unsaturated fatty acids USFA oleic, linoleic, α linolenic, pinolenic, gadoleic, eicosadienoic and sciadonic) and mineral analysis (phosphorus, potassium, calcium, magnesium, sulfur, zinc, copper, iron, manganese, and sodium). Chemical analyses were based on composite samples and performed in triplicate, (Evaristo *et al.*, 2010).



Fig.1. A: *Pinus pinea* pine nuts; B: *Pinus koraiensis* pine nuts (commercial sample).

III – Results and discussion

1. Proximate and vitamin analysis

The proximate composition and vitamin content of pine nuts from 27 *P. pinea* populations grown in Portugal (mean value) along with one commercial sample of *P. koraiensis* nuts are shown in Table 1. For the Portuguese samples crude fat was the predominant component (47.71%). Identical values are found on Mediterranean stone pine nuts from Tunisia (Nasri *et al.*, 2005a, 2005b) and Spain (Cafiellas *et al.*, 2000), but lower (44-45%) and higher (50.3%) average concentrations are also reported, for Turkish (Bagci and Kavaagaçlı, 2004) and Italian pine nuts from *Pinus pinea* (Ruggeri *et al.*, 1998), respectively. In our analysis, *P. koraiensis* presented a higher level of crude fat content (68.07%). Crude protein was the second major component

found in the *P. pinea* seeds analysed with an average of 33.85%. Similar results were reported for other samples of Mediterranean stone pine nuts (Nergiz and Donmez, 2004; Ruggeri *et al.*, 1998; Cafellas *et al.*, 2000). On the contrary, our analyses showed that *P. koraiensis* seeds have a much lower amount of crude protein (14.06%), even lower than that reported previously (17-18%) by Mata (2001). The average moisture for the 27 Portuguese populations was found to be 5.9% while *P. koraiensis* seeds presented 2.5% of moisture, a value far below from that (4.4%) reported by Mata (2001). The present investigation revealed also low levels of starch in Portuguese pine nuts with a mean value of 3.5% DM, when compared with values obtained from chestnuts (43%) (Borges *et al.*, 2008; Künsch *et al.*, 1999). Average starch content of *P. koraiensis* seeds was higher (4.9%) but still registered an equivalent low level. Appreciable amounts of vitamins B1 (thiamine) and B2 (riboflavin) were detected in *P. pinea* seeds, reaching mean values of 0.53 mg and 0.19 mg per 100 g DM respectively. These amounts represented approximately 35.3 and 11.9% respectively of the recommended dietary allowance (RDA), which suggests daily ingestion of 1.6 mg of riboflavin and 1.5mg of thiamin for an adult male (Garrow *et al.*, 2000). *P. koraiensis* seeds revealed vitamin poorer levels, presenting 0.19 mg per 100 g DM thiamine and 0.02 mg per 100 g riboflavin.

Table 1. Proximate composition, riboflavine and thiamine of pine nuts from 27 *Pinus pinea* populations and one *P. koraiensis* sample

	Moisture g/100 g	Starch g/100 g	Crude protein g/100 g	Crude fat g/100 g	Riboflavine mg/100 g	Thiamine mg/100 g
<i>P. pinea</i>	5.9±0.8	3.5±1.1	33.85±1.89	47.71±2.03	0.19±0.25	0.53±0.29
<i>P. koraiensis</i>	2.5±0.0	4.9±0.2	14.06±0.12	68.07±1.22	0.02±0.01	0.19±0.03

Values are the mean value±SD.

2. Mineral analysis

In the present study, the contents of ten minerals were determined (Table 2) in the studied 27 Portuguese populations and the commercial composite sample of Chinese pine nut. Phosphorus (P) (1130 mg per 100 g DM) was the most abundant element in the seeds of *P. pinea* followed by potassium (K) (892 mg per 100 g DM), magnesium (Mg) (533 mg per 100 g DM) and sulfur (S) (485 mg per 100 g DM). The other elements were, in descending order by quantity, calcium (Ca), manganese (Mn), zinc (Zn), iron (Fe), copper (Cu), and sodium (Na). Those results compare favorably with those obtained by Gómez-Ariza *et al.* (2006).

Table 2. Mineral composition of pine nuts from 27 *Pinus pinea* populations and 1 *P. koraiensis* sample

	Macronutrients				
	P (mg/100 g)	K (mg/100 g)	Ca (mg/100 g)	Mg (mg/100 g)	S (mg/100 g)
<i>P. pinea</i>	1130±110.7	892±68.6	31.9±3.0	533.2±38.4	485.4±32.5
<i>P. koraiensis</i>	539±7.51	596±0.2	10.3±0.0	246.3±0.1	201±7.4
	Micronutrients				
	Na (mg/100 g)	Fe (mg/100 g)	Mn (mg/100 g)	Cu (mg/100 g)	Zn (mg/100 g)
<i>P. pinea</i>	1.01±1.25	11.12±1.52	16.05±8.45	3.43±0.29	11.12±0.85
<i>P. koraiensis</i>	0.24±0.05	5.54±0.00	7.34±0.22	1.28±0.08	6.16±0.11

Values are the mean value±SD.

In contrast, our analysis revealed that *P. koraiensis* seeds had a lower mineral content, frequently less than half the mean of *P. pinea* seeds, except for K and Zn. These minerals have many functions in human basal metabolism and are of interest due to their pro-oxidant activity and health benefits (Ozcan, 2006). The mean ratio of Na:K and Ca:P was considered very poor, as it was less than the unity, indicating that mineral supplementation of human dietary is needed (Iqual *et al.*, 2006).

3. Fatty acid analysis

Fatty acid composition of the oil extracted from the seeds is shown in Tables 3 and 4. Nuts are generally recognized for having a healthy fatty acid profile richer in unsaturated fatty acids (USFA). As expected, both *P. pinea* and *P. koraiensis* nut samples contained a predominant fraction of USFA (89.01 and 89.58% w/w TFA), consisting of 39.24 and 29.32% of monounsaturated fatty acids (MUFA) and 49.77 and 60.26% of polyunsaturated fatty acids (PUFA), respectively (data not showed). Saturated fatty acids (SFA) accounted for 10.93 and 7.75% of total fatty acids for *P. pinea* and *P. koraiensis* seeds, respectively. The main contributing SFA was palmitic acid (C16:0) followed by stearic acid (C18:0) in similar proportions relative to the FA total amount for both species.

In agreement with the typical composition of the *Pinaceae* nut oil, the linoleic acid (C18:2) was the most abundant fatty acid in *P. pinea* and *P. koraiensis*. Together with MUFA oleic acid (C18:1n-9), seeds comprised respectively in average 84.76 and 72.2% w/w of total USFA. Although no large differences were found between the two pine species on the linoleic acid content, predominance of PUFA over MUFA was considerably higher (PUFA/MUFA ratio, 2.06 vs 1.275) and the oleic acid concentration distinctively lower on *P. koraiensis* seeds (28.05 vs 38.40%). *P. pinea* and *P. koraiensis* seed oils were also characterized by several minor fatty acids contributing to their composition, such as acids C20:1 *cis*-11 n-9 (gadoleic), C20:2 *cis*-11,14 n-6 (eicosadienoic) and C18:3 *cis*-9,12,15 n-3 (α -linolenic acid, ALA). All studied pine nut oils contained also two particular minor fatty acids of the unusual *cis*-5-unsaturated polymethylene interrupted fatty acid family, namely *cis*-5,9,12 C18:3 (pinolenic acid) and *cis*-5,11,14-C20:3 (sciadonic acid). While sciadonic acid occurs in the seed lipids of all Gymnosperm species containing Δ 5-UIFA, pinolenic acid is restricted to certain families and presents a higher interspecific variation in the genus *Pinus* (Bagcı and Karaagaçlı, 2004). A large discrepancy in pinolenic acid concentration was detected between *P. koraiensis* and *P. pinea* (14.47% vs 0.30%), accounting for its much higher PUFA predominance. On the other hand, *P. pinea* nuts show a minimal level fluctuation in pinolenic acid concentration among all Mediterranean populations analysed so far, from European to Moroccan, Turkish and Tunisian (Nasri *et al.*, 2005 a, b). The same high standards of pinolenic acid in *P. koraiensis* nut oil were reported in other studies suggesting a positive effect on LDL-lowering by enhancing hepatic LDL uptake (Lee, Lee, Lee, Kim and Rhee, 2004), and on appetite reduction by inducing satiety hormones (Pasman *et al.*, 2008).

These results suggest that pinolenic acid is a stable component that could be used to specifically distinguish *P. pinea* from *P. koraiensis*.

Table 3. Saturated fatty acid composition of pine nuts from 27 *Pinus pinea* populations and one *P. koraiensis* sample

	C16:0 %	C18:0 %	C20:0 %	ΣSFA %
<i>P. pinea</i>	6.22±0.17	4.03±0.21	0.68±0.04	10.93±0.27
<i>P. koraiensis</i>	4.98±0.05	2.38±0.04	0.39±0.01	7.75±0.10

Values are the mean value±SD.

Table 4. Unsaturated fatty acid composition of pine nuts from 27 *Pinus pinea* populations and one *P. koraiensis* sample

	C18:1 %	C20:1 %	C18:2 %	C18:3 <i>cis</i> 5,9,2 %	C18:3 <i>cis</i> 9,12,15 %	C20:2 %	C20:3 %
<i>P. pinea</i>	38.36±1.25	0.88±0.07	46.40±1.24	0.30±0.03	0.71±0.06	0.57±0.06	1.79±0.19
<i>P. koraiensis</i>	28.05±0.24	1.28±0.01	44.19±0.00	14.47±0.11	0.00±0.00	0.61±0.00	0.99±0.00

Values are the mean value±SD.

IV – Conclusions

The chemical composition data of Portuguese pine nuts gathered in our study for proximate components, vitamins and fatty acids, is very consistent with the corresponding results of all Mediterranean *P. pinea* populations reported so far, in spite of the geographical differences. We confirmed that pine nuts have high protein, unsaturated fat (mainly linoleic acid) and vitamin (B1 and B2) contents and are a rich source of P, Fe and Zn. As the only exception, mineral composition of Portuguese *versus* Turkish pine nuts showed substantial variations which, upon confirmation by further studies on different producing years and mineral characterization of the remaining origins of *P. pinea* seeds, might prove useful to discriminate Mediterranean populations.

P. koraiensis seeds were characterized in our study by comparatively lower contents of crude protein, moisture and minerals, and higher contents of total fat, in particular PUFA with a particular higher and distinctive fraction of pinolenic acid. Supporting the use of seed FA composition as a *Pinus* chemotaxonomic marker, as suggested by Bağcı and Karaagaçlı (2004), our results suggest that pinolenic acid content is a good biochemical indicator and could be used to clearly distinguish *P. koraiensis* from *P. pinea*. Establishment of tools for kernel identification is particularly important to control the processing procedures, protect producers and avoid unfair competition.

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Toward a traceability of European pine nuts "from forest to fork"

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Abstract. Mediterranean pine nuts from *Pinus pinea* are among the world's most expensive nut species, considered gourmet and health food, due to their rich flavour and excellent dietetic values (35% proteins, 50% fat, mostly omega-6 and -9). But pine nuts industry is very small and locally concentrated, which implies economies of scale rather than economy of scope mechanisms, and a high vulnerability due to a lack of commercial and marketing structures and dependence on one single product and its supply, the annual pine cone yield as wild crop from forests. Major current challenges of Mediterranean stone pine are thus the implementation (and fulfilment) of high quality standards along the supply chain and, very especially, to achieve legal and commercial differentiation from other edible pine seeds that are still sold indistinctly by the generic name "pine nuts": besides *P. pinea*, especially *P. sibirica*, *P. gerardiana*, and *P. koraiensis*. The last one comes from China and holds about 80% of the world market for pine nuts, though it has been marketed in lots mixed and mingled with seeds from other pine species, some of them non-edible like *P. armandii* that causes the Pine Mouth Syndrome, a long-lasting taste disturbance. Recently in June 2012, a new reviewed trading standard was proposed by the UNECE for pine nut kernels, enforcing the uniformity of commercial packages that should contain only kernels of the same species, with the obligate mention of the botanical species name in the product label.

Keywords. Mediterranean pine nuts – *Pinus pinea* – Pine Mouth Syndrome – Food safety – Product labeling – Trade standards.

Vers une traçabilité des pignons de pins européens "de la forêt à la fourchette"

Résumé. Les pignons de pins méditerranéens *Pinus pinea* sont parmi les espèces de fruits secs les plus onéreuses au monde, et sont considérées comme une denrée pour gourmets et un aliment-santé, en raison de leur riche saveur et de leurs excellentes valeurs diététiques (35% de protéines, 50% de matière grasse, principalement omega-6 et -9). Mais l'industrie des pignons est très petite et concentrée localement, ce qui implique des économies d'échelle plutôt que des mécanismes d'économie de gamme, et une forte vulnérabilité due au manque de structures commerciales et de marketing et à la dépendance d'un seul produit et de son approvisionnement, à savoir la production annuelle de cônes de pin comme objet de cueillette dans les forêts. Les défis majeurs pour les pins pignons méditerranéens sont donc la mise en place (et le respect) de normes de qualité plus exigeantes tout le long de la chaîne d'approvisionnement et en particulier l'application d'une différenciation juridique et commerciale par rapport aux autres semences comestibles de pin qui sont encore vendues indifféremment sous le nom générique de "pignons" : en plus de *P. pinea*, figurent notamment *P. sibirica*, *P. gerardiana*, et *P. koraiensis*. Ce dernier provient de Chine et représente environ 80% du marché mondial des pignons, bien que commercialisé en lots mélangés et confondus avec des semences d'autres espèces de pins, dont certaines non comestibles comme *P. armandii* qui est cause de dysgueusie, une altération du goût de longue durée. Dernièrement, en juin 2012, une nouvelle révision de la réglementation commerciale a été proposée par l'UNECE pour les pignons, afin d'uniformiser les lots commerciaux qui ne devraient ainsi contenir que des pignons de la même espèce, et mentionner obligatoirement le nom de l'espèce botanique sur l'étiquette du produit.

Mots-clés. Pignons méditerranéens – *Pinus pinea* – Dysgueusie – Sécurité sanitaire des aliments – Étiquetage des produits – Standards commerciaux.

I – Mediterranean pine nuts, gourmet food from forests

The Mediterranean pine nuts, the edible kernels of stone pine *Pinus pinea* L., are one of the world's top-ten nut species. Pine nuts are currently the most relevant forest product of stone pine forests in the Mediterranean, the forest owner obtaining a higher profit from cone yield than from timber. Moreover, the income is annual and not only in final cuttings at the end of rotation. The mean annual world's pine nut production is estimated in 6.000 t (shelled), virtually all the production still being harvested from the wild. This character as non-timber forest product implies that no specific horticultural techniques are applied and no defined cultivars are used. In the genuine stone pine forests, especially in semiarid Mediterranean climate zones and on poor sandy or rocky sites, the annual mean cone yield ranges from 100 to 1,000 kg/ha, rendering only 4 to 40 kg shelled pine nuts. In more favourable regions like coastal Portugal with a more humid climate, mean production can more than double easily the upper value.

Mediterranean pine nuts are considered gourmet and health food, with a rich flavour and excellent dietetic values (35% proteins, a percentage similar to raw soybeans, and only 50% fat, mostly unsaturated omega-6 and -9 fatty acids). The high prices obtained in the world market, up to 50-60 euros/kg for shelled pine nut as retail price, have motivated the attention of this forest nut by researchers during the last decades. Improvement programmes have been developed aiming to increment the pine nut production and ultimately profitability of forest lands. Clonal selection of plus trees with high cone yield in forests and their evaluation in grafted trials, has allowed for selection of the best-performing clones in each agro-climatic zone, the legal release of superior clones being imminent in Spain for their use in agroforestry systems or grafted orchards (Mutke *et al.*, 2012).

II – Pine species with edible seeds

The pine nuts' character as historically minor forest product implied until few decades ago an insufficient structure of pine cone processing sector, frequently locally concentrated in few villages with a specialised pine nut processing industry, such as in Pedrajas de San Esteban in Spain, Alcácer do Sal in Portugal or Kozak in Turkey, which produce each more than the half of the respective total national pine nut supply. This local concentration implies economies of scale rather than economy of scope mechanisms, and a high vulnerability due to a lack of commercial and marketing structures and dependence on one single product and its supply, the annual pine cone yield, a wild crop collected from forests. Major current challenges of Mediterranean stone pine on international market are thus the implementation (and fulfilment) of high quality standards along the supply chain and, very especially, to achieve legal and commercial differentiation from other edible pine seeds that are sold indistinctly by the generic name "pine nuts". Actually more than 20 different pine species in the world produce edible kernels, besides the Mediterranean pine nut (*P. pinea*) especially the Swiss or Arolla pine (*P. cembra*), its near related red cedar or Siberian pine (*P. sibirica*), whose oil is relevant in the domestic Russian market, the Chinese pine (*P. koraiensis*) that holds about 80% of the world pine nut market, though sometimes mixed and mingled with seeds from other pine species (some of them even nonedible), the Chilgoza or Pakistani pine (*P. gerardiana*), and the American pinyon pines (several species such as *P. cembroides*, *edulis*, *monophylla* and others) (FAO, 1998) (Fig. 1). Virtually all of them are still wild crops, or gathered from rural groves, not from horticultural plantations or orchards, and the limited supply of all of them does not satisfy the increasing demand (Mutke *et al.*, 2012).

On the other hand, the different pine nut species have very different tastes, dietary values (Table 1), and processing quality, thus they are, and must be recognised as, different products that should be differentiated in the market for consumers' security. *E.g.*, true Mediterranean pine nuts double the protein contents of the two other main commercial species, Chinese and Pakistani pine nuts.



Fig. 1. Shelled pine nuts. a.- Mediterranean pine (*P. pinea*); b.- Chinese pine (*P. koraiensis*); c.- Chilgoza pine (*P. gerardiana*).

Table 1. Mean dietary value of shelled pine nuts

Species	Proteins	Fats	Carbohydrates
<i>P. pinea</i>	33–38 %	46–51 %	5–6 %
<i>P. cembra</i>	17–18 %	50–59 %	17 %
<i>P. sibirica</i>	17–19 %	51–75 %	12 %
<i>P. koraiensis</i>	14–18 %	65–68 %	5-12 %
<i>P. gerardiana</i>	12–14 %	51–61 %	20–23 %
<i>P. cembroides</i>	19 %	60–65 %	14 %
<i>P. edulis</i>	14 %	61–71 %	18 %
<i>P. monophylla</i>	10 %	23 %	54 %

Sources: Lanner, 1981; López-Mata, 2001; Montero *et al.*, 2004; Evaristo *et al.*, 2012.

III – The Pine Mouth Syndrome, a serious taste disturbance after consumption of Chinese pine kernels

Pinolenic acid is a specific polyunsaturated fatty acid that is characteristic for *P. sibirica* and *P. koraiensis* seeds, reaching in the latter 12% of total fat contents. On the contrary, Mediterranean pine nuts have a low content of pinolenic acid. This fatty acid stimulates the enteroendocrine system to produce cholecystokinin, a hormone for bile release, used for this reason in dietetics as appetite suppressant. But pinolenic acid has also been related by some researches to the *Pine Mouth Syndrome*, an unpleasant bitter, metallic taste disturbance that can appear 1-3 days after consumption of Chinese pine nuts and lasts for days or even for weeks, sometimes combined with food aversion and other symptoms. Several other hypotheses, still under discussion, are: rancidity due to inadequate processing, storage, and packaging, or, most likely, irritating terpenoid compounds from mingled inadequate, inedible local pine seeds (*P. armandii*, *massoniana*) (Mostin, 2001; Destailats *et al.*, 2010; Munk, 2010; Moeller, 2010; Zonneveld, 2011). Currently, the admixture of *P. armandii* seeds, unfit for human consumption, has been pointed out as the most probable cause of the syndrome (EC, 2011).

Although it has been shown that the Pine Mouth Syndrome (PMS) is exclusively linked to the consumption of pine kernels from China and from no other geographic origins, this food safety issue, long time underestimated by authorities, has had a huge echo in consumers' fora in internet and therefore a negative impact on commerce of all kind of pine nuts, due to the lack of commercial differentiation between species and countries. Moreover, neither EUROSTAT nor Codex Alimentarius (1993) do differentiate pine nuts from different species, and re-exports among countries hamper the traceability of origin and quality of the product. Often, neither the geographic origin, nor even the biological species of imported pine nuts are reported on product labels – therefore they are difficult to trace by consumers in spite of the disparate range of

prices, qualities and the mentioned health issues. Even European nut distributors confound the species, selling Chinese or Pakistani pine nuts indistinctly as true Mediterranean pine nuts, e.g. labelling them in German with the specific product name *Pinienerkerne* that refers to seeds of the species *Pinie*, exclusive name of the Mediterranean stone pine *Pinus pinea*, versus the generic name *Kiefer* or *Föhre* for any other pine species such as the Asiatic ones (Fig. 2).



Fig. 2. Commercial lots of Chilgoza pine nuts (left) and Chinese pine nuts (up) labelled in German incorrectly as *Pinienerkerne* (i.e. true "Mediterranean pine" kernels).

This situation is a clear incompliance with current legal requirements for food labeling and traceability that should cover all stages of food production chains. The *Regulation EC 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety*, approved after the disastrous consequences of the Mad Cow Disease (BSE) epidemic, is based on principles in line with the integrated approach 'From the Farm to the Fork', specifically including transparency, risk analysis and prevention, the protection of consumer interests and the free circulation of safe and high-quality products within the internal market and with third countries (EC, 2002). A recent example of the relevance of this regulation for avoiding foodborne illnesses was the EHEC outbreak in Germany in spring 2011, when back-tracing the food supply chain allowed tracking down the origin of the epidemic to fenugreek seed lots imported from Egypt, discarding other suspects such as Spanish cucumbers.

Without any doubt, also the pine nuts supply chain must fulfil these regulations, identifying the traceable origin of each commercial lot, and especially labelling the correct botanical origin of each product. On this way, recently in June 2012, new reviewed standards were proposed by the UNECE (United Nations Economic Commission for Europe) for pine nut trade, with clear provisions concerning quality, presentation and marking, indicating that commercial packages can contain only kernels of the same species, with the obligate mention of the botanical species name in the product label (UNECE, 2012).

IV – Conclusions

In order to avoid the foodborne illness Pine Mouth syndrome (PMS) and to resolve the failures

of compliance with current legal regulations, national and regional food authorities currently are developing technical standards and public registers for the pine nut supply chain from forest owners, cone pickers, processing enterprises, storages and traders, and the pine nut processing and trading sector itself is increasingly organized in enterprise cooperatives and industry trade associations that promote standardization and technical innovation. On the other hand, traceability improves not only the quality of the product and processes and provides a geographic identification that helps the final consumers to identify the product, but also hinders the still persisting thievery and black-marketing, and it is essential for organic certification labelling.

As roadmap for the Mediterranean pine nuts sector, major issues are the articulation of regional, national and international associations or cooperatives of forest owners, growers and of the pine nut industry, as well as the implementation of regional and national traceability systems for the whole pine nut production and supply chain, and the putting up of high quality standards in production, processing, and trade in European producers. Further targets might be to improve the product profile for a better protection and marketing, e.g. under a "Protected Geographical Indication" (PGI) brand, as well as Traditional or Organic Food Labels, for true Mediterranean pine nuts of each region.

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AGROPINE 2011 Meeting conclusions

The future of Mediterranean Stone Pine requires an effective commercial distinction from other pine seeds and a stable, increased production from agroforestry systems

On 17th-19th November 2011, an *International Meeting on Mediterranean Stone Pine for Agroforestry, AGROPINE 2011*, was held in Valladolid, Spain, organised by the Stone pine sub-network of the FAO-CIHEAM Research Network on Nuts. The meeting brought together about forty experts, researchers, public and private forest managers and land owners, as well as representatives of pine nut processing enterprises from Spain, Portugal, Tunisia, Turkey and Lebanon, with some contributions from France and Chile, in order to review the current state of the art in Mediterranean pine nut production in forests and orchards, and to discuss the challenges of the future.

The pine nut, the edible kernel of the Mediterranean stone pine, *Pinus pinea*, is one of the world's most expensive nuts. Although well-known and planted in antiquity, pine nuts are still collected mainly from natural forests in the Mediterranean countries, and only recently has the crop taken the first steps to domestication as an attractive alternative on rain-fed farmland in Mediterranean climates. The Iberian Peninsula accounts for about 75% of the stone pine area in the world, **Portugal being the main pine nut producer, followed by Spain, Turkey, Lebanon and Italy.**

Over the last century, the Mediterranean stone pine has experienced a range expansion, especially in the Southern and Eastern Mediterranean Basin, as well as a large increase in planted area in its countries of origin, both by forest restoration and farmland afforestation. The species performs well on poor soils and needs reduced cultural practices, it is affected by a few pests or diseases and withstands adverse climatic conditions such as drought and extreme or late frosts. It is light-demanding and hence has potential as a crop in agroforestry systems in Mediterranean climate zones around the world, in tree lines such as shelterbelts adjacent to farmland or pastures or in low density orchard plantations.

The **AGROPINE Meeting 2011** presented the current knowledge and on-going research about ecological and silvicultural aspects of stone pine forests in the Mediterranean basin and the management applied for cone production as one of the multiple forest functions, fully compatible with soil and watershed protection, wildlife conservation, and landscape values. The main technological innovation in the past years has been the generalized use of **tree shakers adapted to stone pines for mechanical cone** harvesting, making manual cone collection by tree climbers – a very dangerous job– an obsolete practice.

Another innovation to increment world production of Mediterranean pine nuts is **planting grafted stone pines**, in specific orchards or in agroforestry systems combined with grazing or farming. Plantations on farmland could yield more pine nuts in the future than the natural forests, contributing to rural development and employment of local communities. This “next step” to domesticate this tree allows the specific use of selected genotypes for higher cone yields, obtained after decades of evaluation in grafted multi-site trials.

The greatest interest during the meeting arose in the **round table discussion and revolved around two major problems of the pine nut sector.** The first challenge will be the **more effective control of cone pests**, especially two native cone-boring larvae, the pine cone weevil *Pissodes validirostris* and the pine cone moth *Dioryctria mendacella*, as well as the Western Conifer Seed Bug *Leptoglossus occidentalis*, recently introduced in Europe from Northern America. Damages caused by these insects reduce cone yield considerably in number and quality, and **effective biological and integrated pest control** would considerably improve the economic benefit from stone pines.

The other major problem of the pine nut as a food item is the **prevailing confusion regarding the identification of the product** among traders, consumers, and even public authorities responsible for the control of the food chain. Pine nuts are among the most expensive nuts of the world, with retail prices ranging from 60 to 80 \$US per kilogram, but at the same time, due to the limited world production, they are a very minor food product in trade volume. There are more than twenty different pine species with edible seeds around the world, though only the kernels of a few major species are traded on international markets, especially Mediterranean, Chinese and Pakistani pine nuts, whereas American pinyon pine nuts are rarely exported. Nevertheless, **pine nuts from different species, or continents, are often not clearly labelled**, or they are even mingled, leading to consumers' confusion, in spite of very disparate tastes and dietary values. No other pine seed has a similar taste to the genuine Mediterranean pine nut from *Pinus pinea*, nor any other is as rich in protein, 35%, a value similar to raw soybeans. Also the processing quality can differ greatly between species, countries and suppliers, as do prices at origin.

Thus, pine nuts from different species are, and must be, recognized as being distinct products and should be differentiated on the market, as an issue of **consumers' rights** and even of **food safety**. This is specially true for Chinese pine nuts, whose commercial lots have been found sometimes mixed and mingled with seeds from other pine species, some of them even non-edible because of their content in irritant terpenoids and other compounds. The consumption of Chinese pine nuts (especially from *P. armandii*) is the origin of the Pine Mouth Syndrome, an unpleasant bitter, metallic taste disorder that can appear 1-3 days after consumption and lasts for days or even for weeks, sometimes combined with food aversion and other symptoms. Beside these consumer's health aspects, the **lack of traceability and correct product labelling, identifying the botanical species and the country of origin, is a clear incompliance with current legal requirements for food labelling and traceability in Europe** (*Regulation EC 178/2002*), based on principles such as transparency, risk analysis and prevention, the protection of consumer interests and the free circulation of safe and high-quality products within the internal market and with third countries. The stone pine supply chain must comply with these regulations.

The follow-up of the 2011 meeting will take the form of the Stone pine sub-network within the **FAO-CIHEAM cooperative research network on nuts**, an inter-regional network made up of CIHEAM and the Regional FAO Offices FAO-REUR (Europe) and FAO-RNE (North Africa and the Middle East). This Network forms part of the European System of Cooperative Research Networks in Agriculture (SCORENA). The next plenary meeting on stone pine is scheduled for 2015 in Portugal.

The abstract proceedings and all communications presented at the AGROPINE 2011 meeting can be found on the meeting web page at: www.iamz.ciheam.org/agropine2011.

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SERIES A: Mediterranean Seminars

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Mediterranean Stone Pine for Agroforestry

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The pine nut, the edible kernel of the Mediterranean stone pine, *Pinus pinea*, is one of the world's most expensive nuts. Although well known and planted since antiquity, pine nuts are still collected mainly from natural forests in the Mediterranean countries, and only recently has the crop taken the first steps to domestication as an attractive alternative on rainfed farmland in Mediterranean climate areas, with plantations yielding more pine nuts than the natural forests and contributing to rural development and employment of local communities. The species performs well on poor soils and needs little husbandry, it is affected by few pests or diseases and withstands adverse climatic conditions such as drought and extreme or late frosts. It is light-demanding and hence has potential as a crop in agro forestry systems in Mediterranean climate zones around the world.

This publication contains 14 of the contributions presented at the AGROPINE 2011 Meeting, held from 17 to 19 November 2011 in Valladolid (Spain). The Meeting aimed at bringing together the main research groups and potential users in order to gather the current knowledge on Mediterranean stone pine as a nut crop and to analyse its potential and current challenges. The presentations and debates were structured into two scientific sessions dealing with management of stone pine for cone production and on genetic improvement, selection and breeding of this species, and was closed by a round-table discussion on the challenges and opportunities of the pine nut industry and markets. Thirty nine scientists, and forest and industry managers, coming from Lebanon, Portugal, Spain, Tunisia and Turkey participated in the meeting, which will hopefully be the first of a series of meetings and activities of the newly restored FAO-CIHEAM Sub-network on Mediterranean Stone Pine.



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