



International Meeting on
Mediterranean Stone Pine for
Agroforestry, Nov. 2011

BOOK OF ABSTRACTS



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The Sustainable Forest Management Research Institute is a joint institute between the School of Agricultural Engineering, University of Valladolid, and the Forest Research Centre CIFOR-INIA. It is a forum for scientific and technical cooperation and coordinated research, innovation, development, dissemination and technical advice in the areas of conservation and sustainable use of forest resources and biodiversity, silviculture and sustainable forest management.

Cover photos

Top left: 3-year old seedling (at 2.600 m a.s.l. near Addis Abeba, Ethiopia, photo W. Tadesse)

Top right: Grafted stone pine at a clone bank 4 year after grafting (Tordesillas, Spain)

Bottom left: Stone pine orchard plantation (Arenys d'Empordà, Spain)

Bottom right: Recent tip-cleft graft, still protected by a plastic bag

Centre: Agroforestry system with stone pine and cereal crops (near Montemor-o-Novo, Portugal)



Time schedule

Thursday, 17th November 2011

9:15 h **The FAO-CIHEAM Interregional Cooperative Research Network on Nuts**

M. Rovira (Network Coordinator), IRTA (Spain)

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

Chair M. Piqué

9:45 h Silviculture of stone pine forests: Models and management tools

R. Calama (Key note speaker), INIA (Spain)

10:15 h The stone pine in Tunisia, history, importance and future prospects

Y. Ammari, INRGREF (Tunisia)

10:30 h Influence of stand structure and silviculture practices on cone and seed production in planted forest of *Pinus pinea* in north of Tunisia

B. Adili, INRGREF (Tunisia)

10:45 h Modelling *Pinus pinea* L. germination in the Northern Plateau of Spain: an ecological-based model as part of a management tool

R. Manso, INIA (Spain)

11:00 h **Coffee break**

11:30 h Climate factors and their relations regarding cone yield of stone pine trees (*Pinus pinea* L.) in Kozak basin

S. Parlak, EFRI (Turkey)

11:45 h Effects of nutrients on conelet losses of stone pine (*Pinus pinea* L.) In Kozak province

S. Parlak, EFRI (Turkey)

12:00 h Modelling spatiotemporal variability in *Pinus pinea* cone production at forest scale

R Calama, INIA (Spain)

12:15 h **DISCUSSION**

13:30 h **Lunch break**

15:00 h Valladolid's province forest land owners: the challenge to optimize pine nut production

M. Rodríguez Blanco, ASFOVA (Spain)

15:15 h Effects of vibration duration and repetitions on efficiency and tree damage of mechanical harvesting for *Pinus pinea* L.

G.L. Blanco Roldán, UCO (Spain)

15:30 h Trunk shakers automation to mechanical pine cone harvesting

M.J. Martín Moya, SEFOSA (Spain)

15:45 h Cone yield evaluation of *Pinus pinea* grafted trial

D. Bono, IRTA (Spain)

16:00 h **OPEN DISCUSSION**



Friday, 18th November 2011

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

Chair S. Mutke

- 9:00 h Population genetics of *Pinus pinea*, a species with low genetic variation.
S.C. González-Martínez (Key note speaker), INIA (Spain)
- 9:30 h Genetic variability for growth traits in the mediterranean stone pine (*Pinus pinea* L.) provenances. *I. Carrasquinho, INRB (Portugal)*
- 9:45 h Release of elite clones of Mediterranean stone pine, *Pinus pinea* L., for cone production in grafted plantations. *S. Mutke, INIA (Spain)*
- 10:00 h Characterization of *Pinus pinea* and *P. halepensis* provenances from Spain and Tunisia related to their rootstock use. *D. Bono, IRTA (Spain)*
- 10:15 h Cloning stone pine (*Pinus pinea* L.) by somatic embryogenesis. *M. Toribio, IMIDRA (Spain)*
- 10:30 h **DISCUSSION**
- 11:00 h **Coffee break**
- 11:30 h **Round table Pine nut industry and markets**
A. Pastor, Piñon-Sol (Spain), F. Pita, Junta de Castilla y León (Spain), P. Silveira, UNAC/ANSUB (Portugal), S. Parlak, EFRI (Turkey), P.R. Sfeir, YMCA (Lebanon)

13:30 h Lunch break

14:30 h Poster sesión

- Dendroecology in Stone pine: a tools for evaluating growth and production models in Cordoba (Andalusia) forests. *R. Sánchez Salgado, UCO (Spain).*
- Effect of stand composition and stocking management in Mediterranean stone pine (*Pinus pinea* L.) forests. *J. de Dios García, INIA (Spain).*
- Early thinning for crown expansion and drought stress reduction. *M. del Río, INIA (Spain)*
- Boron deficiency in stone pines. *J. Bento, UTAD (Portugal).*
- Yields of the mechanized harvest of pine nuts. *P. Martínez Zurimendi, UVa (Spain)*
- Acceleration transmissibility study in *Pinus pinea* L. branches to selective cone harvesting by vibration. *G.L. Blanco Roldán, UCO (Spain).*
- 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. *M. Piqué, CTFC (Spain).*
- Mediterranean stone pine genetic improvement program in Portugal: a methodology for selecting plus trees. *I. Carrasquinho, INRB (Portugal).*
- The forest, a place for sustainable economic development: the YMCA approach to rural development In Lebanon. *P.R. Sfeir, YMCA (Lebanon).*
- Stone pine provenance trials in France, Spain and Tunisia. *S. Mutke, INIA (Spain).*
- Chemical profiling of Portuguese *Pinus pinea* L. nuts and comparative analysis with *P. koraiensis* Sieb. & Zucc. commercial kernels. *I. Evaristo, INRB (Portugal).*
- Toward a traceability of European pine nuts "from forest to fork". *S. Mutke, INIA.*
- Genotype-Environment Interaction and Stability in Thirty-Year Growth of Aleppo pine provenances (*Pinus halepensis* Mill.) in Tunisia. *T. Sghaier, INRGREF (Tunisia).*

16:00 h Stone pine subnetwork follow-up

S. Mutke (Sub-Network Liaison officer) , INIA (Spain)

16:30 h Meeting closure

Saturday, 19th November 2011

8:00 Field trip (Lunch 14:00 h)



The FAO-CIHEAM Interregional Cooperative Research Network on Nuts



Mercè ROVIRA

FAO-CIHEAM Nut Network Coordinator

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The 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 cosponsor the Network. CIHEAM was already involved in fostering research nut tree activities.

The Network structure is based on a Coordination Centre (Coordinator and Secretary), supported by different Subnetworks (Working Groups) having the mission of fostering and coordinating specific activities. IRTA Mas de Bover has been the Coordination Centre from the start of the Network activities, in 1990. Today, the Network has 7 Subnetworks (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 Subnetwork Liaison Officers).

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

- Organization of specific meetings and workshops
- Promotion of R&D activities
- Edition of proceedings and reports
- Edition of inventories of germplasm and research lines
- Edition of the NUCIS Newsletter
- Organization of two international courses on “Nut Production and Economy”
- Training grants for young researchers

Welcome to “Agropine 2011”, the Congress on Mediterranean stone pine, to all people coming from different countries (France, Lebanon, Portugal, Spain, Tunisia and Turkey). This meeting will foster the links between researchers, industrials and potential users of this species. Both Liaison officers of the Stone Pine Subnetwork, Sven Mutke, and the Nut Network Coordinator, Mercè Rovira, invite all of you to participate, discuss and propose different ideas/activities to maintain the Stone Pine Subnetwork alive.



Keynote *Managements*

Silviculture of stone pine forests: Models and management tools

Rafael CALAMA

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Multifunctional character of *Pinus pinea* forests has demanded the attention of Spanish forest managers since the end of XIXth century, when the first forest management plans pointed to a joint optimization of both timber and cone production. The silvicultural practices proposed in that period aimed to the transformation of the forests into normal dense stocking even-aged stands following permanent blocks and clearcutting or shelterwood silvicultural systems. Despite the real interest of fruit production, this management schedule was somewhat biased to optimize timber production. Nevertheless, the relative success of these practices in attaining the proposed objectives of maintaining and improving the forest cover and productions lead to their maintenance up to the 80's of the XXth century. In that period, and as a result of the first international conferences focusing on the species (Madrid, 1989; FAO meeting in Madrid, 1995), a clear demand from forest managers requiring new silvicultural schedules and management tools was identified. This new silviculture should be based on scientific knowledge about the species; be derived from and contrasted on experimentation trials; focus on multifunctional objectives and assume close to nature principles.

In the present work we present a review over the main advances attained in the research line in silviculture and sustainable management of *P. pinea* stands carried since 1992 by the CIFOR-INIA in cooperation with public administrations and other research centers. In this review we first focus on describing the main scientific results, concerning the identification of main factors ruling dynamic and productive processes in the species; the effect of silvicultural practices over these processes; characterization, optimization and economical valuation of products and services; the development of empirical and ecological-based models, or the role of *P.pinea* forests and their management in global change scenarios. Thereafter, we show how these scientific results have been transferred and applied in the daily practical management of *P. pinea* forests.



The stone pine in Tunisia, history, importance and future prospects

Ammari, Y.¹, Piqué M.², Sghaier, T.¹, Solano, D.², Aletà, N.³, Bono, D.³, Hothmani, H.¹, Albouchi, A.¹,
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The *Pinus pinea* is a forest species introduced and naturalized in Tunisia. It was used to stabilize the littoral dunes of Bizerte in the North of Tunisia since 1904 and along the northeast coast in the region of the Cap Bon since 1930. The success of these first plantations incited the foresters to use this species to stabilize the littoral dunes of the northwest, too. Again, the success of the *Pinus pinea* plantations in the dunes of Saouania, Ouechtata, Béchateur and Rimel encouraged the foresters to use this species to reforest clearings degraded in northwest cork oak forest. Indeed, it is between 1967 and 1974 that stone pine was used as species of reforestation for the ligneous production in Mogods and in Kroumirie in mixture with *Pinus pinaster*, *Eucalyptus sp.* and cork oak. Its area of distribution in Tunisia includes the wet and sub-wet climatic zones. In these bioclimatic fringes, the pine showed itself acclimatized well to the local conditions and gave interesting production in spite of the variability of the pedological and climatic conditions. Surfaces occupied by pure stone pine stands or in mixture with other species are of the order of 35,000 ha. At present, thanks to the success of the former plantations and the high demand of the pine nut by the population, this species became the most used in the programs of reforestation after the Aleppo pine. Given that the difference in age of the plantations, the Tunisian *Pinus pinea* plantings were divided up on two categories. A first category trained by the old populations (Dar Chichou, Rimel) whose vocation can be only the wood production, and the second category trained by more recent plantations (age less than 40 years) which can be directed for the production of pine nut by subjecting them to appropriate silvicultural treatments.

By referring to some studies made on the growth and the production of this species in Tunisia, the ligneous production was considered in approximately 3-9 m³/ ha/year, whereas that in cones in approximately 50-250 kg/ha/year according to the station fertility. Tries of vegetative multiplication by takings of cuttings and by grafting of *Pinus pinea* were introduced during 1970, but which were fast abandoned. In 2008 and within the framework of a Tunisian-Spanish project of cooperation, aiming at the improvement of the social conditions of the rural forest populations (living inside and in the bordering forests zones), the grafting of *Pinus pinea* on *P. pinea* or *P. halepensis* rootstocks were led to accelerate its precocity of fruiting and spread its area of reforestation on basic grounds. The first results obtained within the framework of this project are encouraging. So, during the first year of the project, rates of 22 % success were obtained by the grafting on stone pine and 27 % success by the grafting on Aleppo pine stocks. During the second year, rates of success of the order of 43 % were realized. These encouraging results can be improved by improving the quality of the grafted seedlings and the control of their cultural conditions in the nurseries.

Keywords: *Pinus pinea*, littoral dunes, reforestation, ligneous production, pine nut, grafting, nursery, seedlings quality, rate of success and cultural conditions.



Influence of stand structure and silviculture practices on cone and seed production in planted forest of *Pinus pinea* in north of Tunisia

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Pinus pinea L. is one of the most valuable species used in the Tunisian reforestation program for its ecological, aesthetic and economic value. It was successfully introduced at the beginning of the 20th century along the Mediterranean coast line. Today in Tunisia it occupies an area of 21,000 ha. Despite this importance, little is known, at least in Tunisia, about fruiting, cone yield, variations in seed morphology and quality of *Pinus pinea* and how these different processes are affected by the stand structure and silvicultural practices. It was reported that some factors such as high crop year, overgrazing and illegal harvesting influence seed production.

Variation in cone size, seed number per cone, seed viability, seed quality and seed germination behaviour relative to stand conditions (age, diameter at breast height, height, crown diameter, stand density, social status of tree) were analyzed for three *Pinus pinea* stands in Mekna III, located in north coastal dunes in Tunisia. The cones were collected in winter 2011 on sample of 10% of trees in DBH classes, counted and weighted.

Three cones with different sizes (small, medium and large) for each sampled tree were taken and brought to the laboratory. The length and the width (at the widest point) of the cones were measured with vernier callipers, the weight was also determined. Afterwards, seeds were released by heating the cones at 60 °C for 30 mn to 1 hour to open them. Number, size and total weight of seeds per cone were determined. Damaged or not well-developed seeds were recorded, and their viability relative to germination was tested.

We will provide sources of variability in cone and seed production relative to stand density, age, social status of trees and silvicultural operations. However the data show that seed production has been low to extremely low, whatever the considered stand. 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. Therefore proper management and regulation of livestock grazing and cone collection in the *Pinus pinea* forests is mandatory to both sustainable nut and forest productions.



Modelling *Pinus pinea* L. germination in the Northern Plateau of Spain: an ecological-based model as part of a management tool

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The Northern Plateau of Spain accounts for over 50,000 ha of managed *Pinus pinea* forests. A remarkable effort has been devoted to model pine nut production throughout a wide range of silviculture alternatives and climate scenarios. Those studies provide a valuable tool to optimize seed production through a suitable management scheme. However, present regeneration treatments (shelterwood method) face a common natural regeneration failure, implying extra investments to achieve it by direct seeding. In order to better understand the underlying ecology driving the process and to propose efficient regeneration treatment schedules, a model comprising all phases of natural regeneration is being developed. In this study, we present the corresponding germination submodel.

Our experimental design consists of two 40 m x 60 m plots installed in 2006, in a representative *P. pinea* stand, under two different felling densities. From 2006 to 2010, 50 pine nuts were placed at the beginning of August inside 18 cages within each plot. Cage location was established to account for three levels of insolation and two pasture types, with three replications each. Number of germinants was recorded fortnightly for 12 months or until total germination took place. Germination modelling was ecology-based approached through lifetime analysis of censored data. In order to accomplish this task, we defined a hazard germination function depending on time varying (climate) and time independent, spatial variables, on a daily basis. Integration of all daily risks of germination prior to a particular time is the probability of a seed to germinate at this time. Temporal/spatial data correlation was taken into account including random effects.

Results show that daily probability of germination is climate-mediated. Spatial variables depending on the tested stand densities resulted non-significant. Optimum conditions for germination were in the vicinity of a daily maximum temperature of 15 °C and high levels of relative humidity. On the other hand, frost occurrence proved hampering for the process, exhibiting certain time persistence. Given the typical meteorological year in the area, predicted germination favourable conditions represent a rare narrow window arising during fall and spring seasons. Providing that seed dispersal take place during the warm period and maximum predation rates are associated to winter, fall germination could be one of the key factors for successful regeneration events. These findings suggest that higher flexibility in regeneration felling schedules should be considered. This will allow enough favourable events to occur in order to guarantee natural regeneration.

Keywords: Natural regeneration, forest management, survival analysis, time-dependent variables



Climate factors and their relations regarding cone yield of stone pine trees (*Pinus pinea* L.) in Kozak basin

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This study aims to reveal the factors that affect cone yield of both natural and planted stone pine trees (*Pinus pinea* L.) in Kozak Basin, Bergama, Izmir - Turkey.

In the basin, productive and non-productive areas have been determined, and according to long term observations it is found that there is no significant difference regarding vegetative development of the trees, therefore climatic factors have been investigated whether they have a key role. For that purpose productive and non-productive areas have been marked on 1/25,000 scale topographic maps and verified with actual studies in field. After studies it has been determined that productive areas are above certain altitude and then possible factors which can affect yield have investigated.

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 and data obtained from these stations for years 2009 and 2010 have been associated with pollination periods.

According to phenological observations, pollination periods are between 12-20 May at altitude 230 m (non-productive), between 28 May and 3 June at altitude 430 m (non-productive) and between 19-31 May above 500 m (productive).

Productive areas have lower relative humidity and higher wind speed than non-productive areas. In non-productive areas number of days temperature goes below $-10\text{ }^{\circ}\text{C}$ and relative humidity rates over 70% have been found higher than productive areas. As a result of low wind speed, relative humidity increases, pollens stay suspended in air and pollen transfer to conelets does not occur.

It has been determined that during pollination period a wind speed of 3 m/s or more are very effectual in pollination. Also determined that pollen movement is related with wind speed and relative humidity, and wind speed is main factor in opening of pollen tubes and for pollen distribution. It has been found that productive upper basin of Kozak has enough wind speed, in non-productive areas number of windy days and wind speed are not sufficient.

Likewise it is understood that pollination also is influenced negatively from precipitation occurring during pollination period, high relative humidity of air and extremely high and low temperatures. Also it is highly possible that high relative humidity during this period causes fungal diseases.

In non-productive areas it is determined that there is a temperature difference reaching to $22\text{ }^{\circ}\text{C}$ between day time and night, early and late frosts frequently occur. 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*



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

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Kozak Province is situated in Bergama District in Izmir. Basin has a bowl shape and an area of 16,000 hectare of stone pine forest which is the one-third of the total stone pine area in Turkey. Almost all forest area is private.

The bedrock of the Stone pine forests' area is granite. Soil of the area is in coarse textured classes. Soil textural classes are sand, loamy sand and sandy loam. Soil reactions (pH) of whole areas are between 5.76 and 6.89. It means that soil reactions are slightly acidic and neutral. Soils of the whole area are non-saline and limeless.

In recent years, conelet losses of stone pine have been increased. For this reason, 8 different areas were examined to determine the relations between conelet losses and nutrients during 2005 – 2008.

Almost all cone losses occurred in new conelets according to the results of cone counting in the sampling area. Therefore, evaluations were based on this period. In the cone losses, significant differences were found between years and regions.

To determine the relation between cone losses and nutrients, 1, 2 and 3 ages needles were taken from the sample trees and measured as their size and weight (fresh and dry), then analyzed in terms of micro and macro nutrients. Soil samples were taken beneath the same sample trees and analyzed.

A correlation analysis was applied for examination of relation between cone losses and needle size, fresh and dry weight. According to results, a negative correlation was found between cone losses and needle size, needle weight (fresh and dry). That means, smaller needle size, lessen fresh and dry weight and increased cone losses.

By the examination of relation between cone losses and the plant nutrients (macro and micro) in needle, a negative relationship between cone losses and nitrogen, phosphorus, calcium and manganese have been determined. In other words, when these nutrients diminished, cone losses increased. It is considered that, among these elements, especially phosphorus and calcium combined with drought could be a factor in the cone losses.

Also, a negative relationship between altitude and conelet losses was determined. Lower altitude increased cone losses, but no linkage between altitude and conelet formation (occurrence).



Modelling spatiotemporal variability in *Pinus pinea* cone production at forest scale

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Accurate predictions of the annual cone production at different spatial scales are a basic requirement for the sustainable management of stone pine (*Pinus pinea* L.) forests in the Spanish Northern Plateau. Forest managers demand tools helping them to quantify the annual cone crop in order to fix the annual initial price for public auctions of cone cropping, to identify most and less productive areas within the forests and to incorporate cone production into the forest management planning. Moreover, the forest owners are interested in knowing the expected crop of cones for their forests in a given year, in order to optimize crop campaigns. Finally, the cone transformation industry would benefit from this knowledge since it permits them to guarantee and plan in advance the supply of raw material.

In this work we present an application of the tree level scale spatiotemporal model for cone production (Calama et al. 2011, *Ecological Modelling* 222, 606-618) to predict spatial and temporal variability at a forest scale. As a case study we propose to use the Public Forest 44, “*Tamarizo Nuevo*”, a 1,000 ha stone pine forest located in the sand lands of the province of Valladolid. The data from the management inventory, observations from the cone crop in previous years and the available meteorological data were used to calibrate the model and to predict cone production for the crop campaigns 2011-2012, 2012-2013 and 2013-2014. As annual variability is explained by climate events occurred during the three years before cone maturation, the level of uncertainty in the predictions increases for the farther predictions, since non-observed meteorological data must be substituted by expected random values based on the historical meteorological series.



Valladolid's province forest land owners: the challenge to optimize pine nut production

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Mediterranean stone pine has in Valladolid one of the most important references all over the world. Not only the main pine nut industry located in Pedrajas de San Esteban but also the almost 65.000 ha of natural and planted forest, most of them still need of specific management for cone production, must lead the agents related to work together.

Particularly efforts must be focused on private forest land owners, most of them characterized by no profitable small owned areas . Especially relevant is the case of the areas planted thanks to the CAP politics, without a medium-large term planning. This lack of management both in natural and planted areas has in the association of forest land owners a great chance to be solved. Through the temporal grouping of small areas is possible to execute forest works focusing of pine nut production and to celebrate auctions in order to get better and more stable prices for the pine cone harvest.

Important efforts must be also developed in research knowledge transfer. The great range of research work done must be accessible and understandable by private owners because they owned the 60% of forest land areas and the future production depends of them.

The forest land owners association *Asociación Forestal de Valladolid (ASFOVA)* works since 1987 on the lines marked before. More than 600 forest land owners have in ASFOVA a reference for consulting new management techniques, selling their harvest and requiring information to establish new plantations. In the last years, ASFOVA has also lead a great effort to get the current harvest and marketing regulations and to make owners and pine cone pickers to obey them.

Forest land owners call the restored FAO-CHIEAM Inter-Regional Cooperative Research work of nuts to take into account their needs that in short are the needs of the future of pine nut.



Effects of vibration duration and repetitions on efficiency and tree damage of mechanical harvesting for *Pinus Pinea* L.

Gregorio L. BLANCO-ROLDAN, Sergio CASTRO-GARCIA, Jesús A. GIL-RIBES

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Traditionally, stone cones are harvested manually. The worker, aided with tools, climbs to the tree and selects the mature pine cones with a consequent high labour risk and a high harvesting cost. Currently, due to these economic reasons and worker safety conditions, mechanical harvesting with trunk shaker is replacing hand harvesting methods. Trunk shakers have been designed for agricultural crops and their regulation and operation system are not adapted to stone pine requirements.

In this study, an experimental trunk shaker was tested in order to determine the effect of different vibration parameters, vibration durations and repetitions on the mechanical harvesting result. Trunk shaker adaptation was focused on increasing the harvesting cone efficiency and reducing the tree damage by vibration -shoot and unripe cones detachment and bark damage-. Field tests were carried out in two forests located in the south of Spain during two harvesting seasons.

Trunk shaker regulation using a frequency range of 16-19 Hz and trunk acceleration values of 55-75 m/s² reached a mature cone harvesting efficiency with a mean value of 85.7%. Less than two non-harvested organs per tree were detached with these vibration parameters. Harvesting date was found to be a main parameter to avoid tree damage, mainly bark damage. It was recommendable not to shake the tree after the growth beginning of new shoots to control the potential damage caused to the tree. Vibration duration up to 4 seconds was not useful to increase mature cone detachment. Also, two short time duration vibrations were effective to increase harvesting efficiency without increasing tree damage.



Trunk shakers automation to mechanical pine cone harvesting

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The lack of skilled labour to cone harvesting, the high wages paid and the unacceptable safety conditions in which they work have promoted endless attempts to mechanical harvesting, using mainly olive trunk shakers, even though they are not designed for this job.

The use of trunk shakers for cone harvesting requires its automation to avoid damage to the bark of the trunk as well as remove unripe cones and even shoots. In addition, trunk shakers automation makes it possible to optimize the cone harvested process, resulting in an increased performance and a better percentage and quality of removal.

This study shows the development of a new automation system compatible with most trunk shakers on the market. The system consists of a set of mechanical, hydraulic, electrical and electronic components that mounted on a trunk shaker makes it possible to control accurately the parameters that characterize the vibration process: height and angle with the trunk of the shaker clamp, grasp pressure and duration and intensity of the vibration. Moreover a flow regulator was inserted into the hydraulic circuit, capable of modifying the rotational speed of its eccentric mass and therefore the acceleration and frequency values applied during the vibration.

Among the components of the system it can be included a telematics module that makes it possible to create a register of traceability that contains information supplied by the sensors the system includes, and can be accessed in real time and remotely.

Field test with a trunk shaker available in the market were performed in the mountains of Huelva (south of Spain), using different frequencies and vibration times. The quality and efficiency of the mechanical harvesting were evaluated along with the operation system.

The results confirmed the potential of the system, which simplifies significantly the job of the operator, who only has to drive the tractor and face the shaker to the tree before starting the automatic process.



Cone yield evaluation of *Pinus pinea* grafted trial

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Pinus pinea grafted plantations for fruit 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 genotype and more adapted rootstocks to the soil, easily harvesting, better control against the fire, etc.

Some *P. pinea* grafted plantations and experiences have already been done; however their productive information is still scanty. The oldest ones correspond to reforestations transformed by grafting. However, their cone yield evaluation was discontinuous and consequently provided imprecise data. More recently, genetic studies, directed to select productivity in *P. pinea* genotypes, have been done using grafted pines on the clonal banks of C.N.M.G.F. Puerta de Hierro (Madrid).

With a view to go into the species agronomical potential knowledge in depth, in 2008 *P. pinea* grafted trial was planted at the IRTA Torre Marimon Center (Caldes de Montbui, Barcelona). The planting distance was 6 m x 6 m, occupying a total surface of 0.35ha; the soil has a low fertility with sandy texture and lightly alkaline pH. Grafted plants, grown up on containers, were planted (*P. pinea* scion, from Cataluña litoral provenance, onto Aleppo pine rootstock). Drip irrigation was installed and weed competence has been controlled. In 2010 an irrigation essay started, in order to study the avoidance of the natural *P. pinea* forest alternate bearing. Two irrigation treatments were established, considering different application periods, as well as one control without irrigation. The trial design was on randomized complete blocks, with three repetitions and two trees per observation. Growth, phenological features and production were recorded. To evaluate the effect of different water regimes on the floral induction and productive responses (flower and fruit mortality, fruit quantity and quality) was the main objective of this irrigation essay.

The first results on the trial show an early onset on production of trees, from barely 50 flowers/ha in 2008 to more than 3500 produced in 2011. In 2009, trees produced, about 1800 flower/ha, the first significant flowering year, which around 85% kept as cones on the tree in September 2011. Concerning the hydric supply only preliminary results have been arisen. A lower fruit mortality on the second development year (7% against 20%, in 2010) and a greater floral induction (14 flower/tree against 10 flower/tree, in 2011) of the irrigated trees against those without hydric supply (control) can be pointed out.

With the further results, productive potential of this kind of plantations is expected to be evaluated, establishing a guide for maximizing the production and improving cone quality as well.



Keynote *Genetics*

Population genetics of *Pinus pinea*, a species with low genetic variation.

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The European stone pine (*Pinus pinea* L.) constitutes an exceptional example of a genetically depauperate but geographically widespread plant species. Genetic variation is considered a prerequisite for adaptation and colonization of new environmental conditions. Stone pine challenges this basic principle. This talk will focus on recent findings from putatively neutral molecular markers, thus providing information on the evolutionary history of the species and the current standing levels of genetic variation. Both organelle (chloroplast microsatellites) and nuclear (nuclear microsatellites and candidate genes) markers found very little molecular variation in *P. pinea*. To provide a baseline for comparison with *P. pinea*, we searched the literature to identify other cases of genetically depauperate plants. This search indicated that *P. pinea* is truly exceptional among widespread, sexually reproducing plant species for its low level of genetic diversity. This low genetic diversity, on the other side, allowed the development of a simple test to differentiate *P. pinea* from other commercial pinenuts (i.e. those from *P. koraiensis* and *P. gerardiana*). The current pattern of population structure shows two main, highly differentiated gene pools, in the eastern (Greece, Turkey, Israel, Cyprus and Lebanon) and western (Spain, France, Italy, Morocco and Tunisia) Mediterranean. Assuming that observed variation does not represent new mutation, stone pine appears to have passed through severe and prolonged demographic bottlenecks, with more stable populations at the edges of its distribution, followed by subsequent natural- and human-mediated dispersal across the Mediterranean Basin. The stone pine case study illustrates that genetic diversity, as measured by marker loci, is not necessarily correlated with high potential for adaptation.



Genetic variability for growth traits in the

Mediterranean stone pine (*Pinus pinea* L.) provenances

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Provenance trials provide information on adaptability, growth and yield of the species. These studies have great practical use in the improvement of forest stands, as it can assure sources of seed that give well-adapted, productive trees in reforestation and afforestation.

In 1992, a Mediterranean stone pine provenance trial was initiated in Portugal. Twenty-eight seed lots, from seven different countries throughout the species' native range were obtained with the cooperation of Silva Mediterranea network. In February 1993, twenty-five provenances (five from Portugal, three from Spain and Italy, two from Greece and Israel, four from Morocco and six from Turkey) were established at "Perímetro Florestal de Sines" (38° 01' N Lat., 8° 42' W Long.), fifteen (6 from Turkey, three from Italy, two from Portugal and Morocco and one from Spain and Greece) at "Mata Nacional de Valverde", Alcácer do Sal (38° 19' 30" N Lat., 81° 32' W Long.) and twenty (seven from Turkey, five from Portugal, three from Italy, two from Greece and Morocco and one from Spain) at "Perímetro Florestal da Conceição", Tavira (37° 12' N Lat., 7° 39' 55" W Long.). Unequal distribution of seed sources within the three experimental sites was related to different seed germination per seed lot. In 1998, Alcácer do Sal field site was abandoned because of the high mortality provoked by unexpected flooding. Tavira field site was also lost during the 2003 forest fire.

The aim of this study was to detect genetic variability for the Mediterranean stone pine. Total height was used as an indicator of provenance variability. Mixed models were applied to data at different ages obtained from those three field trials. Results showed that, at least for a significant level of 0.05, provenance variability for height was found to be significant.



Release of Spanish elite clones of Mediterranean stone pine, *Pinus pinea* L., for cone production in grafted plantations

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Due to its aptitude to grow in poor soils and semiarid climates, the Mediterranean stone pine, *Pinus pinea* L. has more than doubled its area in the last two centuries as results of protective or restoring afforestations, reaching currently about 0.7 million hectares around the Mediterranean Sea. Its cones have been recollected by man since Palaeolithic for their edible seeds, the Mediterranean pine nuts, today one of the most expensive nuts of the world. But little effort has been made for its proper domestication as a nut crop: no defined cultivars are known and nearly all the current stone pine nut production is harvested from forests where no horticultural techniques are applied. Only in the last decades increasing efforts have been made in clonal selection for pine nut production in grafted orchards.

In Spain, in the last twenty years, about 500 hectares of grafted stone pine plantations has been planted by forest administrations or private land owners, pure or mixed with cork oak in an innovative multipurpose system that will combine high-value non-wood forest productions (cork, pine nuts) with soil protection and grazing facilities for livestock or wildlife. In 1989, several governments of Spanish autonomous regions started the prospection of plus trees in the pine forests, based on an elevated individual cone production, aiming at their propagation in grafted orchards.

Since 1990, grafted clonal trials have been established including some 500 candidate clones from different regions. Twenty years later, the analysis of individual yield series in these trials has allowed the selection of the best performing genotypes for their release to the forest sector and private land owners as basic materials for production of forest reproductive materials (scions for grafting), aiming at their commercial use for pine nut production in agroforestry. The result is a catalogue of elite clones for grafted orchards in different agroclimatic zones in Spain. In 2012, their legal release as tested basic materials for graft scions is expected in Spain. Similar advances exist in Portugal.

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

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On the last years some experiences of grafted *P. pinea* as a fruit crop have been done. The evaluation of their productive potential or the selection of the best productive genotypes were the main objectives. However, there are few experiences related to the rootstocks. Currently, there are serious doubts to choose the best adapted material in each environment or about the rootstock effect on phenological features and fructification.

Behaviour trials of different *P. pinea* and *P. halepensis* provenances under controlled conditions have been done on the framework of PCI project, managed by CTFC and INRGREF, with IRTA and CIFOR-INIA collaborations. The aim was to make an early characterisation of those materials to know which rootstocks are better adapted to the environments of new *P. pinea* plantations. So, in 2010 a controlled essay with seedling (1+0) of 8 Spanish and Tunisian provenances was carried out at IRTA, Centre Torre Marimon. Four of them were Aleppo pine provenances ('Kef', 'Thibar', 'Sallent' y 'Palau-Sator') and the other four Stone pines ('Remel', 'Mhibes', 'Aiguafreda' y 'Caldes de Malavella'). Seedlings were planted on Coneplast C-20R containers of 2.5 liters. Half of them filled with peat and vermiculite (1:1), for the rest a mixture of peat, vermiculite and sand (1:1:1) was used. Three water stress levels were applied: 20%, 10% and 5%, which were the minimum volumetric contain (cm³ water/cm³ substrate), respectively, used. Experimental design was in split-plot with three replications and 20 plants per observation unit.

Data were recorded in three different periods (beginning, middle and ending of the trial). Plant height, plant diameter and allocated biomass of each portion (below and aboveground) was measured to calculate total biomass and the ratio root/shoot. Moreover, physiologic parameters (RWC and CT) and ontogenical differentiation information (adult needle proportion and winter buds formation) were noted down. WUE_i was estimated from the $\delta^{13}\text{C}$ analysis of some control plants. On the following years, this nursery characterization will be complemented with a field trial network. These materials, as rootstocks, will be established on different edaphic and climatic conditions in Spain and Tunisia.

High intraspecific variability and a more clear response to the water stress treatments and also to the different substrates have been observed in *P. halepensis*, with the first results. Under stress conditions, 'Thibar' provenance showed the lowest allocated biomass reduction and the highest root/shoot ratio. On the other hand, 'Kef' and Tunisian *P. pinea* provenances displayed a significant decrease of the total biomass, with the increase of water stress level. Tunisian *P. halepensis* provenances had the lowest RWC values, in both favourable and stressful scenarios. They showed higher precocity on juvenility loss, as well, especially in the case of 'Kef' origin. The $\delta^{13}\text{C}$ results placed the 'Palau-Sator' and 'Sallent' *P. halepensis* provenances on the highest and the lowest WUE_i position, respectively. The rest of Tunisian materials were ranged in intermediate positions, independently of the species.



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

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Plant propagation is one of the major issues in tree breeding and genetic resource conservation. The development of biotechnologies of propagation such as somatic embryogenesis (SE) 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. Plant regeneration by 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. 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*, 1985), SE has been achieved for many pine species as *P. pinaster*, *P. radiata*, *P. strobus* and *Pinus 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.

We reported plant regeneration by somatic embryogenesis in Stone pine, and further research is in progress to improve the efficiency of the several steps. SE was obtained at a 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 plant. The establishment of suspension cultures offer technical advantages over semisolid cultures. Liquid cultures produce faster growth rate, and allow 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. We have characterized the embryogenic suspension cultures of Stone pine, and growth rate of ESM and somatic embryo morphology has been evaluated. The effect of the liquid culture conditions on maturation ability is also being analysed.

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Dendroecology in Stone pine: a tools for evaluating growth and production models in Cordoba (Andalusia) forests

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The impacts of climatic conditions in Stone pine growth rate in drought-prone areas and their relationship with cone production have not been properly evaluated taking into account the differential responses to climate change of local sites, time, tree and stands conditions. We evaluated these responses assessing the changes in radial growth and production of plantations of Stone pine (*Pinus pinea*) in Sierra Morena region (Andalusia). We used dendrochronological methods and mixed models of basal area increment to quantify the responses of trees to site, stand and climatic conditions. In the study area, a minimum temperature rise and a decrease in spring and fall precipitation have led to drier conditions during the late twentieth century which culminated with a sequence of severe droughts (1994, 1995, 1999 and 2005) affecting directly to Stone pine growth and production.

Comparisons with the climatic records showed that both the radial growth and cone production were influenced positively by (fall) spring precipitation in the (previous) current year and negatively by winter minimum temperature. The Principal compound and correlation analysis showed three main components (climate, structure and physiography) related with growth and production, respectively. High growth rate was associated with tree size (dbh), competition (low density), site conditions (north aspect) and climatic conditions (fall precipitation and winter minimum temperature). The local production model showed positive relationship with crown diameter, fall-winter precipitation, and minimum winter temperature; and negative with stand density, north aspect and slope.

The annual variation in production could be explained largely by the climatic conditions during the three previous years of the collected year. The relationship between growth and climate in the three previous years could be implicated in the mature process of cones. The growth rate and widespread cone production of Stone pine plantations in Córdoba could improve their future knowledge with the study of moving correlation between climate, stand structure and fixed factors like physiographic conditions.

Key words: basal area increment, dendrochronology, Mediterranean climate, mixed models, *Pinus pinea*, plantation, production.



Effect of stand composition and stocking management in Mediterranean Stone pine (*P. pinea* L.) forests

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Mediterranean forests are among the most vulnerable ecosystems under climate change scenarios. Thus measures to manage these forests according to the expected reduction in total and summer rainfall and to the increase in temperatures are necessary. The use of thinning to reduce stocking density and the promotion of mixed stands have been proposed as effective measures for adaptation. Thinning is assumed to improve stand water balance and water availability by reducing competition. Assuming an optimal spatial distribution of trees and species in a mixed stand, each individual is expected to occupy the most favourable niche for growth. Under these assumptions, trees in low dense mixed stands are expected to show a better response in terms of growth than trees growing in pure high stocking stands.

This work shows the devices and techniques used to evaluate the role of stand composition and stocking management in the adaptation to climate change scenarios in stone pine forests of the Spanish Northern Plateau. The previous hypotheses were contrasted for Mediterranean stone pine (*Pinus pinea* L.) by assessing the diameter increment in a 5 years growth period (id5), in three different stocking management scenarios corresponding with basal areas of 10, 15 and 20 m²/ha. Trees were grown either in pure even-aged stands or in mixed *P. pinea* – *Juniperus thuriphora* - *Quercus ilex* heterogeneous stands. Pure and mixed stands were selected within the same region, covering a wide range of stocking densities, individual tree sizes and inter- and intraspecific competition status. Id5 was assessed using data from radial increment cores covering the 5 year series 2004-2008. Comparisons were done by fitting linear models including dbh, stocking density and species composition as explanatory variables for predicting id5. Our results showed a greater effect of stocking density than species composition.

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Early thinning for crown expansion and drought stress reduction

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Silviculture in stone pine plantations in Spain includes traditionally an early pruning, aiming to promote apical dominance, even since the first thinning. However, the effect of pruning on crown architecture is not yet verified. In the same way, although the effect of thinning on diameter growth is well studied, there are less information concerning crown dimensions. Previous studies indicate that thinning can mitigate the effect of drought, but all studies focussed mainly on diameter growth. In this study we evaluate the effect of pruning and thinning on tree development, analysing diameter, height and crown variables. The study was carried out in a pruning and thinning experiment located in Valladolid (Spain). The experiment was established in a 10 years old stone pine plantation following a complete block design with three blocks and two factors, pruning and thinning, with two levels (yes or not), involving a total of 16 plots. 10 trees per plot with similar initial competition status were selected to study the tree development. Diameter, height and several crown attributes (crown diameter, shoot growth and inclination angles of lateral branches, etc.) were recorded from 2006 to 2010. From July 2006 to December 2010 monthly diameter growths were also registered for studying the phenology of secondary growth.

Trees showed a positive response to thinning in diameter, height and crown projection area growths, while the effect of pruning was only statistically significant (negative) on diameter growth. Thinning favoured diameter increment with statistical differences in most of the months, but the greatest differences during the summer drought period. Its positive effect on height growth was remarkable, especially after the severe drought of 2009, given that thinning usually does not affect height growth. This suggest that the control of stand density in early stages, eliminating suppressed trees and improving tree vigour, is key measures to avoid extreme drought effects as crown diebacks and productivity reduction. Early stand density reduction by thinning stimulated the diameter growth and crown expansion of residual trees. This hastens the start of cone production, since crown development favours onset of female reproduction (21% of the trees in thinned plots, 15% in control plots). Regulating stocking density eliminates suppressed trees and improves tree vigour, a key measure to avoid extreme droughts effects, such as crown diebacks (12% of the trees affected in control plots, only 4% in thinned). Early pruning did not improve crown architecture and did not favour apical dominance. Therefore, it should be delayed until after the second thinning for reducing considerably treatment costs. Thinning is an useful measure for adaptation of stone pine plantations to climate change, particularly in Mediterranean environments where more frequent and more severe droughts are expected.

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Boron deficiency in stone pine

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Pine stands are widely visible in Portuguese forestry areas. *Pinus pinaster* is the most dominant species occupying about one third of the total forest lands; in the whole country other pine species are also largely represented, such as the typical Mediterranean/Continental *Pinus pinea* and *P. halepensis* or the Atlantic/Mountainous *Pinus sylvestris* and *P. nigra*.

P. pinea is classified as an indigenous species in the most Mediterranean climate influenced regions of the country, which are mainly located inland and along the central and southern coast.

Several forestry projects carried out with this species, in the interior part of the country often failed, resulting either in the initial death of seedlings or in poor growth of the remaining plants. Poorly shaped young trees with needles presenting chlorotic symptoms show repeated terminal necrosis buds, with loss of apical dominance and multiple lateral shoots, which gives them a shrubby aspect. Such inaptitude has usually been explained as the result of extreme climatic conditions with icy winters and very dry summers. The similarity between such appearance and boron deficiency symptomatology has led to further evaluation of the nutritional state of these plants. The results obtained from leaf nutrient analysis have increased the evidence of a boron deficiency.

A 4x4 randomized blocks field experiment was implemented in such a situation, testing the efficiency of 0, 2.5, 5.0 and 10.0 g/tree fertibor® (14.8% B) application. In a short period of 6 months the visual evidence of boron deficiency had disappeared in the treated plants, presenting also higher diameter and height increments. Seven years from the beginning of the experiment, the height of the treated plants doubled high significantly ($p < 0.001$) the untreated ones.

Empirical evidence of boron deficiency in large tracts of reforestation initiatives, in diversified locations, allows inferring about the species sensitivity concerning the mineral nutrition failure of this element.

Key words: stone pines; reforestation; boron deficiency



Yields of the mechanized harvest of pine nuts

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The use of tree shaker is currently the main system for pine cones harvesting system, thus studies of times and efficiency of these machines are needed for the planning of work and the calculation of costs. This study analyses the time spent by the tree shaker that work in the forests of the province of Valladolid, in each activity of the harvest of cones, through two techniques of timing, discontinuous and continued. Also the factors on which depend these times and yield have been analysed: the experience of the operator and the type of the used machine. With discontinuous timing two machines have been analysed: the telescopic forklift Manitou 730-120 LS and compact equipment Sicom 200. The statistical analysis was made of the comparison of means. On the machine Sicom 200, the harvesting time, yield, and efficiency were compared when the machine operator has experience of more than one campaign or without experience (less than three months of handling). With the technique of timing continued the time spent in each activity, and yields of 15 machines have been analysed: 8 tractors, 4 telescopic forklift, 2 light compact machine and 1 wheel loader. The average length of each operation of the work cycle was: displacement 10.28 seconds, positioning 3.82 seconds, embraced the tree 2.91 seconds, vibrating 3.77 seconds and withdrawal of the clamp 4.24 seconds. The average duration of the complete cycle, that is to say, the average time used for each tree is 27.06 seconds. The machines that more time took to complete a cycle were light compact machine (39.64 seconds), followed by telescopic forklift (31.17 seconds), wheel loaders (29.79 seconds) and tractors (27.49 seconds).



Acceleration transmissibility study in *Pinus pinea* L. branches to selective cone harvesting by vibration

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Pinus pinea L. cones take three years to mature while other newer pine cones are growing on the tree. Nowadays, hand harvesting is the main method for harvesting full-grown nuts. However, mechanical harvesting by trunk shakers, usually used in olive and other agricultural crops, is being used in this forest specie without previous studies or regulations. In order to reduce the tree damage and increase the mature cone detachment is necessary to adjust the vibration parameters used by the trunk shakers. Machine regulation could contribute to the implementation of good harvesting practices and improvement of the sustainable production of nonwood forest products.

The aim of this study is to determine the pine cone response under forced vibration in *Pinus pinea* L. branches, under controlled laboratory conditions, to evaluate the suitability of stone pine cones for selective mechanical harvesting by vibration. 26 branches were tested featuring cones of three different ages. Branch excitation was applied using an unidirectional electromagnetic shaker with a random noise with frequencies from 0 to 60 Hz, for a total time length of 60 seconds. Three piezoelectric triaxial accelerometers were used to measure the acceleration on the branch base, cone branch junction and over the mature cone. A total of five main frequencies with high vibration transmissibility values were identified from the branch base to the cone base.

In relation to mature cones, significant differences were detected in the frequencies with the fourth and fifth highest values (16.74 and 25.64 Hz, respectively) and with vibration amplifications 3 times greater than the input vibration. The study of vibration transmissibility reveals that in mature cones, a single maximum value is produced at 18.00 ± 5.28 Hz, where resonance phenomena can appear. The morphological changes produced in the cones from the third year, mainly in their stalk and total weight, influenced vibration transmissibility for the detachment of ripe cones, lowering the importance of the vibration transmissibility along the branch and increasing the importance of the stalk system for selective mechanical harvesting.



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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We introduce the 4-year project (2008-2011), from 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 main objectives of the project have been: a) to strengthen institutionally INRGREF in order to promote its research and training capacities with regard to production and management of grafted stone pine; b) 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; c) to assess grafting success on different vegetative materials and grafting techniques; d) to evaluate the adaptation of different stock to variable hydric and edaphic nursery conditions; e) to produce plant stock from selected Spanish and Tunisian vegetative materials for the establishment of new plantations; f) to implement field trials in both countries, on different hydric and edaphic conditions, for evaluating the development of the plantations and g) to share and transfer techniques and results.

During the first year of the project (2008) the research teams came together to update available knowledge and reinforce capacities for vegetative materials collection, also was done an implementation in Spain of a first grafting trial in nursery.

In 2009 focus was done 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 by means of a visit of the Spanish team to Tunisia. A field trial under controlled conditions with Spanish grafted material was also done.

In 2010 the trials implemented in Spain during the previous years were followed. In Tunisia a nursery was built and equipped, in order to implement further trials and to produce suitable grafted materials. Despite grafting success kept being low, the systematic methodology is consolidating and Tunisian team can start training local staff. A further selection of vegetative materials and new nursery experiences are performed.

During 2011, field trials under natural conditions are to be implemented, and during the AgroPine2011 meeting an internal seminar will be held for sharing experiences and results emerging from the project, which may enhance research and transfer capacities.



Mediterranean stone pine genetic improvement program in Portugal: a methodology for selecting plus trees

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In Portugal, the Mediterranean stone pine (*Pinus pinea* L.) occupies approximately 118,000 hectares, which represents 3.5% of the total forest area. The total area devoted to stone pine has increased since the last inventory by about 51%, which is due to an increased interest in the species by forest land' owners. Increasing kernel production, in terms of quantity and quality, is one of the main purposes of this species genetic improvement program. To assure the sustainability of the breeding program, selection should include young and/or small trees, which must be the most productive for their age and size. The identification of different developmental groups, accomplished by gathering trees with similar dimensions and cone production, is a way to guarantee unbiased plus tree selection, especially in uneven-aged stands, which constitutes the prevailing situation in Portugal.

In this context, multivariate statistical analysis (cluster and discriminant analysis) was used to define developmental stages for stone pine, considering tree size and cone production without site-specific information, and to assign trees to the five different obtained. These levels were: juvenile (stage 1), dominant vegetative growth (stage 2), cone production phase I (stage 3), cone production phase II (stage 4) and cone production phase III (stage 5). The methodology to select plus trees involved only the productive stages 3, 4 and 5.

Some trees were identified as candidate to plus trees, as their individual annual cone production was among the highest within each development productive stages. However, the authors sustain that these trees must be evaluated yearly for their cone production for at least seven years, period constituting the average productive cycle. Besides, the stone pine development stages approach allows that any unclassified tree can be assigned to each developmental stage, applying the discriminant functions developed.



The Forest, a place for sustainable economic development: the YMCA approach to rural development in Lebanon

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In Lebanon, the forest cover is estimated at 13% of the country's total surface (136 900 ha) consisting of broadleaves, coniferous and mixed forests. The broadleaved forests cover the majority of the Lebanese forests 57% mainly of *Quercus* (*Q. calliprinos*, *Q. cerris*, *Q. infectoria*) followed by the coniferous (32% of forest area) mostly of pine where *P. brutia* and *P. halepensis* are the dominant species; the *P. pinea* forests are 36% of the coniferous forests and were mainly planted between 1930 and 1940. The mixed forests 11% are fragmented and scattered: stands of mixed pines, mixed pine and cypress and mixed conifers plantations. Other types of trees are also found like fir, cedar, cypress, carob, ash, laurel, poplar, willow etc. Despite the fact that the Lebanese forest code restricts the exploitation of timber and non timber forest products from the coniferous forests, the only product under exploitation is the pine nut from the *P. pinea* representing thus the main source of income for the rural communities in 80% of the middle mountainous zone. The potential of the pine nut industry is still at its primary phases and requires assistance especially that forest users have contributed, based on their own expertise, to the management of the pine forest to meet its economic potential.

The YMCA (Young Men's Christian Association) in collaboration with the Ministry of Agriculture and through the technical guidance of the CTFC (Centre Tecnologic Forestal de Catalunya) and the financial support of the AECID (Spanish Agency For Cooperation And International Development) has developed a forest management project for the *P. brutia* forest in Lebanon. Being the second most abundant forest species in Lebanon, the *P. brutia* forests are highly threatened by fires due to the lack of proper management. The project's main objective is to develop a scientific tool for the establishment of sustainable forest management strategy through: generating new information on the forest and its resources (data collection and inventories), developing decision support tools (growth models) and the capacities of the governmental entities (trainings and workshops). The collected data from 50 plots of *P. brutia* is used to develop a model that supports the decision making process for the sustainable management of the *P. brutia* forests. The second phase of the project is carried out in a pilot forest in Northern Lebanon. Its main objective is to connect the forest to the neighboring communities offering potential economic opportunities for the sustainable management of the forest; such as eco-tourism, aromatic plant drying, and biomass heating systems. The following will lead to the establishment of a forest association grouping all stakeholders able to take in charge the management, conservation and protection of the forest and its resources.

This project is a pilot of its kind in Lebanon and the Middle East. It presents an innovative approach to the development of the rural communities through the forest management. The successful partnership established between the different stakeholders involved in the forest whether public/private owners, the Ministry, the forest users, is entitled to put into practice the results of the scenarios generated by the model. The innovative approach followed within the *P. brutia* project proved to be successful and replicable to other types of forests provided the methodology is adapted to the specific context. Currently, the methodology is being replicated for the Oak forests (*Q. calliprinos*). *Pinus pinea* forests are potential candidates for they can easily adapt to a multipurpose management system. However, the fact that the majority of the Stone pine forests are privately owned is a challenge by itself.



Stone pine provenance trials in France, Spain, and Tunisia

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Mediterranean Stone pine (*Pinus pinea* L.) is a native species widely used for reforestation in the Mediterranean for soil protection, ecosystem restoration and both for pine nut and timber production. It is characterised by a near absence of genetic variation at both phenotypic traits and molecular markers, and a strong phenotypic plasticity that allows for high survival rates in severe environments. In the early nineties, experimental plots were established in the framework of FAO *Silva Mediterranea* and French INRA in several countries bordering the Mediterranean Sea for studying the distribution of genetic variability between provenances in adaptive traits such as survival, vegetative and reproductive phase change, phenology and growth. Here we report the results of the first fifteen years at nine trial sites in France, Spain and Tunisia, other plots existing in Portugal, Turkey, and Morocco. In Spain, four test sites were analysed (Tordesillas, Quintos de Mora, Cucalón, and Trespaderne), three sites in southern France (Le Trepas, La Gaillarde, and Sainte Baume), and two in Tunisia (Jbel Essmaa and Oued El Bir).

No significant differences in survival or ontogeny were found between accessions, while height growth differed significantly between provenances, though this genetic variability was masked by a common, stable reaction norm in dependence on site and microsite that produced strong spatial autocorrelations of tree height, both between and within sites. After nearest-neighbours adjustments that captured the spatial patterns, the significance of the geographic genetic variation increased considerably, in some test sites it became significant only after this adjustment.

Comparison of the adjusted average heights of each provenance showed a common trend between sites. At the Spanish test sites, most provenances from inner Spain and one from France achieved the highest growths, whereas especially coastal provenances from southern Turkey, southern Spain and the French Côte d'Azur grew less. Applying a Principal Component Analysis of the mean tree heights for 29 provenances at the four Spanish test sites only the first component was retained, accounting for 65% of the observed variance, and correlating significantly with the mean annual temperature in origin as climate proxy for each provenance ($r = -0.68$). The colder provenances did not only grow more, but showed also an earlier spring flush and higher tendency to form Lammas shoots. Analysing the only 7 provenances present at all test studied sites in the three countries, the first principal component accounted still for 55% of the variance, with nearly the same negative correlation with temperature in origin ($r = -0.67$). Currently, the trials enter reproductive maturity, and the analysis of these data will allow new conclusion about possible adaptive variability.



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

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Stone pine (*Pinus pinea* L.), being endemic to the Mediterranean Basin, obtains an extreme economical relevance in Spain, Portugal, Italy, Tunisia and Turkey, where pine nuts are traditionally marketed and consumed.

The Portuguese area of stone pine forest is estimated in 130,000 hectares. Due to the species plasticity, *P. pinea* can be found throughout the country. Amongst the seven provenance regions delineated in the Portuguese territory, Provenance Region 5 (South of Tagus) gathers about 62% of the total area due 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.

In recent years 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.

The purpose of this work was to determine the detailed composition and nutritive value of pine nut, assessing geographically distinct populations to investigate potential differences in their profiles. Considering that the origin is an important issue for producer and consumer protection, this study aimed also at finding parameters that could distinguish *P. pinea* nuts from Chinese pine nuts (*P. koraiensis*).

Based on the analysis of 27 different populations, pine nuts were characterized by high contents of fat (47.7g per 100g dry matter DM), protein (33.8g per 100g DM) and phosphorus (1130mg per 100g DM) and low contents of moisture (5.9g per 100g DM) and starch (3.5g per 100g DM). They were also found to be a good source of zinc, iron and manganese. A comparative analysis with *P. koraiensis* nuts showed lower protein (14.1g per 100g DM) vitamin and mineral contents, and higher fat (68.1g per 100g 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 kernels of these two species. A significant variability was found in nut composition of Portuguese populations, but no distinct geographical differentiation was detected.



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

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The forest and plantations of Mediterranean stone pine, *Pinus pinea* L., cover about 0.7 Mha around the Mediterranean Sea, half of which are the result of afforestations since 1850. This tree was also introduced, mainly as ornamental, in other bioregions (California, Chile, Argentina, South Africa, Australia, New Zealand). Since Palaeolithic it has had relevance in local cultures, for timber, fuel, and for its large, edible seeds: the Mediterranean pine nuts, one of the world's top-ten nuts. They are the main forest product of the stone pine forests, rendering higher annual revenue. Depending on environment and applied silviculture, the mean annual cone yield is about 100-1,000 kg/ha in forests, i.e. 1-40 kg (4%) shelled pine nuts. The high retail value obtained for pine nuts (50–60 €/kg) and their fragrant flavour make them one of the most expensive nuts of the world and a genuine gourmet food. On the other hand, they are health food, containing 35% proteins, being rich in omega-3 and -6 fatty acids and trace elements. In the last 20 years, genetic improvement has been undertaken based on clonal selection of plus trees with high cone yield in forests, and their testing in grafted orchard trials in different agro-climatic zones where the use of stone pine in agroforestry is considered. The mean annual cone production of stone pine in agroforestry systems is expected to exceed 1,000 kg/ha, registering in grafted orchards cone yields up to 2,000-3,000 kg/ha. These prospects were one of the reasons to organise the present AgroPine2011 meeting.

But there are more than twenty different pine species with edible kernels. The most relevant kinds of pine nuts on the world market are obtained in Europe from the Mediterranean stone pine (*P. pinea*) and more locally the Swiss or Arolla stone pine (*P. cembra*), in Asia from *P. koraiensis* in China and Corea, the chilgoza pine *P. gerardiana* in Pakistan, and *P. sibirica* in Siberia, and in America from several pinyon pines, such as *P. cembroides* or *edulis*. Virtually all of them are still wild crops, or gathered from rural groves, not from horticultural orchards, hence the limited supply does not satisfy the increasing demand. All of these pine nuts have very different tastes, dietary values, and processing quality, i.e. they are in fact different food items. In spite of this, the different pine seeds are often not marked as such in the commerce, nor recognised as different species by the consumers. This sometimes intentional confusion is also, but not only, a question of disparate world market prices that depend on the species and processing quality: e.g. cheaper Chinese pine seeds are sold in Europe often for the (fourfold) retail price of true Mediterranean pine nuts. But it implies also serious health risks. e.g. the notorious Pine Mouth Syndrome caused by the ingestion of Chinese pine nuts: an unpleasant bitter, metallic taste disturbance, that appears 1-3 days after consumption and lasts for days or even for weeks, sometimes combined with food aversion and other symptoms. Several hypotheses for its causes are still under discussion, such as: rancidity due to inadequate processing, storage, and packaging, irritating terpenoid compounds from mingled non-edible pine seeds (*P. armandii*, *massoniana*), or high contents of pinolenic and other rare polyunsaturated fatty acids that cause enteroendocrine disturbances. Thus, the correct labelling and traceability of pine nuts, just as any other food, is a food safety issue, besides the commercial interests of Mediterranean pine nuts producers and consumers rights that should be defended. The strict application of the European Regulation (EC) 178/2002 about requirements of food law and procedures in matters of food safety "...from farm to fork" must hence be claimed for pine nuts and should be one of the main targets of the European and Mediterranean pine nut industry.

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Genotype-Environment Interaction and Stability in Thirty-Year Growth of Aleppo pine provenances (*Pinus halepensis* Mill.) in Tunisia.

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A multisite experiment was conducted in Tunisia in 1964 to study the genotypic performances of forty local and foreign Aleppo pine (*Pinus halepensis* Mill.) provenances. Five morphological characters were measured: the total height, the diameter at breast height (DBH), the density of wood measured on and under bark and the general shape of trees. The analyses of variance done after thirty years of growth on the common provenances to the 4 experimental sites indicated the presence of significant genotype-environment interactions. These interactions were studied using the joint regression (FINLEY et WILKINSON, 1963 ; EBERHART et RUSSELL, 1966 ; PERKINS et JINKS, 1968 ; SKRØPPA, 1984), the WRIKE (1962) ecovalence and the Hühn (1979) parameter of stability. The obtained results showed that the two sites of Nâam and Souiniet, considered as representative of the extreme conditions of growth for the species in the country, were contributed with more than 75% to the interaction for total height and DBH characters. The decomposition of the interaction according to provenances' showed that the three foreign provenances: the Moroccan provenance (A) and the two Italian provenances (AJ and AL), as well as the Tunisian provenance (L) are at the origin of most of this interaction, especially for the growth and shape characters. The three first provenances being adapted to the adverse environment conditions, while the Tunisian provenance is adapted to the favorable environment conditions. The parameters of the wood density are in opposition to the growth parameters. Finally, by taking account of the performance of the tested provenances and the obtained results for the adaptability and stability parameters calculated for each measured character, the four Tunisian provenances AG (Selloum), Q (Semmama), R (Berino) and Y (Mellègue) can be regarded as the most powerful and most stable for growth (total height and diameter) and form characters.

Key words: *Multisite experiment/ Pinus halepensis/ provenances/ interaction/ adaptability/ stability.*



Seminar Proceedings

The Seminar Proceedings, including the text of the keynote lectures, oral presentations and posters accepted by the Scientific Committee will be published in the CIHEAM journal *Options Méditerranéennes*. Articles may be written in English or French, with a maximum length of 8 pages in format 17x24 cm.

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Guidelines for manuscript, template for writing articles and an example of article are available at the [meeting website](#).

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