NUCIS
NEWSLETTER

Information Bulletin of the Research Network on Nuts (FAO-CIHEAM) Number 9 December 2000

CIHEAM

IRTA - Mas Bové • Coordination Centre of the Research Network on Nuts

Institut de Recerca i Tecnologia Agroalimentàries

FAO CIHEAM Nut Network

EDITORIAL

Almond tree blooming in Tarragona, Spain

Activities 2000

During the year 2000 some important activities were carried out within the FAO-CIHEAM Interregional Cooperative Research Network on Nuts following its programme (1997-2002). The First International Symposium on Stonepine was held in late February in Valladolid, Spain. This Symposium was organized jointly by Junta de Castile and Leon, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA) and the Confederación de Organizaciones de Selvicultores de España (COSE) together with the collaboration of another ten organizations, the FAO-CIHEAM Nut Network included.

Genetic resources inventories

Regarding the Inventories on Germplasm, Research and References, the second Inventory on Hazelnut, following the first on Almond, was published after being edited by A.I. Köksal and various collaborators. This inventory published as a REU Technical Series, is an important compilation of the currently available hazelnut genetic resources and information on on-going research projects and bibliography. In addition, three more inventories are being compiled and are at different stages of completion. The Inventory on Chestnut, edited by G. Bounous, is almost ready, the Inventory on Walnut being compiled by E. Germain is close to completion and the Inventory on Pistachio is being collated by N. Kaska and B.E. Ak. All these catalogues are being funded by FAO's Regional Office for Europe and the Seed and Plant Genetic Resources Service (AGPS) together with CIHEAM-IAMZ.

Network restructurization

The current FAO-CIHEAM Research Nut Network structure is based on nine subnetworks (see back page of the issue) and the Coordination Centre. This structure is regarded as too extensive. During the Ninth Meeting of the ESCORENA Coordinators held at the Institut National Agronomique Paris-Grignon (INA, P-G), 23-25 November 2000, the simplification of the structure of the Network to make it more compact was discussed. This suggestion has been made several times as the Network is expensive to run. Originally in 1989, the Network was structured on five crop-based subnetworks: almond, hazelnut, pecan, pistachio and walnut. The subnetworks on Chestnut and Stonepine were established in 1991 and 1995, respectively. Two disciplinary, rather than species oriented, subnetworks were added in 1994: Economics and Genetic Resources. Five Coordination Board meetings have already been held (Spain, 1991; Turkey, 1994; Portugal, 1995; Morocco, 1996 and Italy, 1997). During the Fifth Coordination Board Meeting held in 1997 in Rome, it was decided that the following meeting (the Sixth Coordination Board Meeting) should be held in 1999 where steps on this issue would be taken. However, this meeting has been postponed until 26-28 April 2001, to be held in Zaragoza, Spain and the renewal process still stands. There is an ever-increasing lack of funding to organize the Coordination Board meetings for discussion and planning of future activities. In addition, due to the forthcoming retirement of some liaison officers, it is necessary to undertake a process of change and renewal. Largely the process would be a reduction in the number of subnetworks and joint management.

Response to the NUCIS 8 questionnaire

A questionnaire requesting information about the FAO-CIHEAM Research Nut Network and this Newsletter was attached to the NUCIS 8 issue. In addition, in order to update our database, there was a section to be filled with the complete address, telephone, fax and electronic mail address and field of interest (al-

Kunsch U., Scharer H., Patrian B., Hurter J., Conedera M., Sassella A., Jermini M., Jelmini J., 1999. Quality assessment of chestnut fruits. Proc. 2nd Int. Symp. on chestnut. Acta Hort. 494: 119-127.

Marinoni D., Boccacci P., Akkak A., Bounous G., Ferrero A., Botta R., 2000. Impiego di marcatori microsatelliti di quercia per lo studio del germoplasma piemontese di castagno (*C. sativa* Mill.). Atti giornate scientifiche SOI, Sirmione, Brescia.

Mc.Carthy M.A., Meredith F.I., 1988. Nutrient data on chestnut consumed in the United States. Economic botany, 42: 29-36.

Merz F., 1919. Die Edelkastanie: Ihre volkswirtschaftliche Bedeutung, ihr Anbau und ihre Bewirtschaftung. Verlag Schw. Departement des Innern, Bern. 71 S.

Muti E., 2000. Il cibo e il nostro corpo e il cibo e il nostro peso, ovvero la Bibbia delle calorie, Demetra, pp. 495

Panatta G.B., 1999. Un frutto energetico e gustoso. Il Divulgatore, Bologna, XXII, 10: 73-76.

Rasmussen T.S., Henry R.J., 1990. Starch Determination in Horticultural plant Material by an Enzymatic-Colorimetric procedure. J. Sci. Food Agric. 52:159-170.

Senter S.D., Payne J.A., Miller G., Anagnostakis S.L., 1994. Comparison of total lipids, fatty acids, sugars and nonvolatile organic acids in nuts from four *Castanea* species. J. Sci. Food Agric. 65:223-227.

Zhou J.R., Erdman J.W., 1995. Phytic acid in health and disease. critical reviews in food. science and Nutrition.35.495-508.

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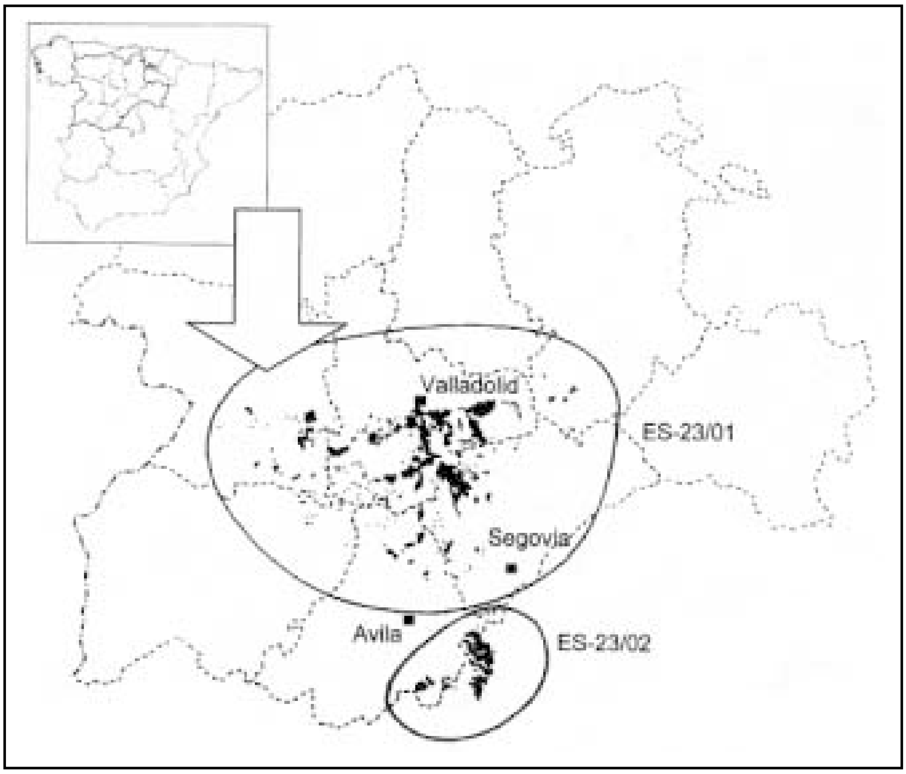
THE STONEPINE (*Pinus pinea* L.) BREEDING PROGRAMME IN CASTILE-LEON (CENTRAL SPAIN)

Figure 1. Stonepine areas in Castile-Leon.
ES-23/01: Meseta Norte, ES-23/02: Tietar and Alberche Valleys



THE STONEPINE IN CASTILE-LEON

The stonepine, *Pinus pinea* L., is one of the most characteristic tree species of the Mediterranean Basin due to its singular umbrella shape. Since the antiquity, it has been part of the landscape and was used as nut tree, since its cones produce large edible kernels, known in Spanish as "piñones".

In Spain, the stonepine forests cover some 400,000 ha, two thirds of the world's total stonepine area; 176,000 ha of them have been deemed as natural stands. Main growing areas are the Southwest (Western Andalusia) and the "Meseta Norte" (central highland plateau in Castile-Leon), but pure or mixed stonepine stands spread also over other areas in Catalonia, Castile La Mancha, Madrid or Extremadura. In order to regulate the use of forest reproductive material, seven provenance regions and four limited local areas have been defined in Spain, based on geographic and ecological criteria (Prada et al., 1997).

In Castile-Leon there are two provenance regions. Table 1 exposes a short ecological characterization of the two provenance regions and Figure 1 shows their stonepine areas. "Meseta Norte" (number ES-23/01) includes the 50,000 ha in the central Douro Basin, the main part being natural stands. They are part of the "Tierra de Pinares" (Pine Country) between Valladolid, Segovia and Avila. This country is a vast, sandy plain covered with 170,000 ha of mixed or pure maritime pine (*Pinus pinaster* AIT.) and stonepine forests. Stonepine dominates in the province of Valladolid, where it is the main forest tree species and occupies 42% of the total forest area of the province (Spanish Forest Resource Survey 1995). The geographic region "Meseta Norte" is a sedimentary plain surrounded by high mountain ranges, which separate it from the mild oceanic influence. Therefore the climate is continental with marked hot dry summers and long winters. Occasional late frosts can occur up until May or June and early frosts from October. The distribution of rain is irregular as much as between years as between seasons, except the three months of summer drought.

Since pre-Roman ages, the predominant land use has been for dry farming agriculture, with grain as main crops. In the central province of Valladolid, 80% of the land surface is cultivated, and only 13% remains as forests which have not been broken up. This is mainly because of its minimal agronomic aptitude. Specifically, the pine forests are relegated to sandy areas in the south of the Douro valley - authentic dune areas -, to calcaric lithosols and to clayey or gypseous marls in the easily eroded slopes.

The second provenance region in Castile-Leon (ES-23/02) Tietar and Alberche Valleys, spreads over the limit areas of the provinces of Avila, Madrid and Toledo. They are situated in the southern foothills of Gredos and Guadarrama mountain ranges in a genuine Mediterranean climate. The main function of these forests is soil, wildlife and landscape conservation. This is due to their situation on hills and mountain slopes and the presence of endangered or endemic species such as Iberian lynx (*Lynx pardina*), Iberian eagle (*Aquila adalberti*) or black vulture (*Aegypius monachus*).

In former times, the pine forests provided the local population with a large number of resources, documented since the 12th century (Gil, 1999). Nowadays, the main functions of the remaining forests are defined as environment protecting and conservation of the ecosystem, its biodiversity and its underlying genetic diversity. At the same time, the open and luminous stonepine forests are the preferred outing and picnic areas for the urban population. The cone yield is currently the most important commercial production of the stonepine forests and provides average yearly incomes of 24 euros per hectare[1] to the owner (in the most productive stands up to 96 euros/ha yearly), whereas the timber sale comes only in second place (15.5 euros/ha annual). Other marketable products and uses, as grazing, fuel wood or hunting, which are minor.

The local pine kernel industry

The increasing economic importance of the pine kernel production results in sustained incomes for the pine forest owners. Also the regional kernel market has consolidated in the village of Pedrajas de San Esteban (Valladolid). The average value of stonepine cones sold in tree[1] is more than 400,000 euros only for the public forests of Valladolid province, the third part of “Meseta Norte” stonepine stands. The pine-kernel shelling and manufacturing industry in the “Tierra de Pinares” area includes about seventy companies, with a turnover of 12,000,000 euros and processing 1,500 to 2,500 t of unshelled kernels originated from Castile-Leon, Andalusia, Castile La Mancha and Portugal.

Grafted stonepine plantations

Among the nut trees, the stonepine remains as being one of the most genuine forest species, as it was never domesticated in cultivated plantations like other crops. At present the cones are still harvested in forest stands. The difficulties of its cultivation are one of the possible reasons of this lack of Neolithic revolution.

Stonepine clone growing at the “Meseta Norte” genebank

The stonepine does not root as cutting, nor does it allow woody grafting. Scions should be obtained from long shoot terminal buds at the very moment of initiating its spring flush, or from other soft tissues like needled dwarf shoots. Furthermore, the pruning possibilities are limited, as the pine does not form long shoots from axilar buds, but only from the terminal buds whirl at the top of the branches. Another point against the stonepine cultivation is the three-year-long wait for maturation of the cones. This implies that three consecutive yields coincide in the tree, so a very irregular fruitfulness between years results. Finally, female conelets and male catkins are situated separately in different parts of the tree’s crown. During the first 10-15 years the grafted stonepines behave like female individuals without male flowers, and need to grow near to mature stands that act as pollinators. In conclusion, the stonepine is not an easy crop.

But perhaps the main reason to discard stonepine as cultivated crop has been the reduced kernel output. Some 200 kg of cones, average harvest per hectare of natural stands, will give some 40 kg of shelled kernels, and only 10 kg of unshelled kernels. Furthermore, until the first steam driven shelling machine was designed at the end of 19th century, kernel shelling one-by-one was a manual labour of women and children. So the costs of kernel harvesting, transport, extraction and shelling made the pine kernel more a pastries ingredient or luxury speciality than a genuine food that could match up with other crops’ efficiency as interesting farmland use.

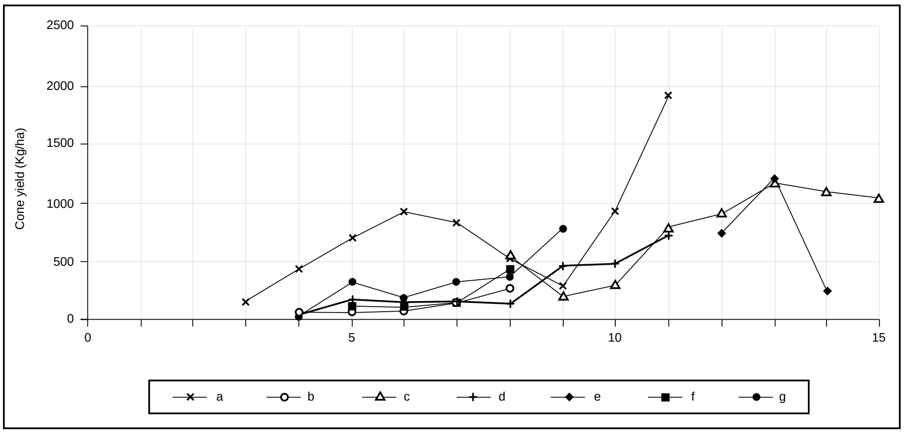
Actually, there is not any report about the use of grafted stonepines before the

Table 1. Provenance region of *Pinus pinea* L. in Castile-Leon (Prada et al., 1997)

Provenance Region	Altitude (m)	Climate variables (range)			Lithology		Stonepine Area
		T(ºC)	P(mm)	sp	rock	pH	(approx. Ha)
1. Meseta Norte	650 - 900	10,5-13,5	351-610	2 - 16	s k	6,4-7,0 7,6-8,8	50.000
2. Tietar and Alberche Valleys	600-1000	13,2-15,1	648-1007	7 - 12	s	5,7-7,0	3.500

T = temperature (ºC); P = rainfall (mm); sp = minimal monthly rainfall in summer (mm); s = siliceous parental rock; k = limestone rock.

Figure 2. Review of cone yield reports in grafted plantations (calculated for 278 trees per hectare)
a - Monte Tudia y sus Faldas, Badajoz (Garrote, 1994, cit. in Mutke et al., 2000)
b - Clone bank, Italy (Magini & Giannini, 1971, cit. in Mutke et al., 2000)
c - Pezuela de las Torres, Madrid (Catalán & Catalán, 1996, cit. in Mutke et al., 2000)
d - El Carrascal, Valladolid (Gordo et al., 2000)
e - Valdegalindo, Valladolid (Gordo et al., 2000)
f - Puerta de Hierro, Madrid (Sada et al., 2000)
g - Cabeza Aguda, Cordoba (Abellanas et al., 2000)



1960[th], apart from local use in the village of Biar (Alicante), where grafting stonepine on wild grown *P. halepensis* rootstocks (Cavanilles, 1797, *cit.* in Prada et al., 1997). Since the 60[th], Italian breeders have carried out grafting experiments, to propagate selected stonepine clones, and few years later the experimentation started in Spain too.

Thirty years of experiences later, Prada et al. (1997) and Catalán (1998) exposed the currently known advantages, possibilities and yields of grafted stonepine plantations. Here we only emphasize as the main advantage of grafting in new stonepine plantations, the early coming in production at few years[2]. Opposited to it, traditional stonepine stands do not start to yield cones until about twenty or thirty years later. The economic viability and profitability of these plantations is analysed by Abellanas et al. (2000) and Mutke et al. (2000). Based on data of experimental plantation (Figure 2), the results of these papers show that the potential use of grafted stonepine can be a very interesting alternative in farmland afforestations.

General lines of the stonepine breeding programme in Castile-Leon

As a consequence of the change in profitability of the different products of pine forest, currently timber yield comes into second place. Cone yield is the main marketable production of the forest, managed under the paradigm of protections and multiple use. Therefore, the improvement of the stonepine will have to combine three objectives. Genetic resource conservation in the silviculture and management of the existing natural regenerated forests, specific treatments for cone yield improvement in the most productive stands, and a defined breeding line orientated to obtain and propagate high productive genotypes for their use in new plantations and farmland afforestations.

The first two items result in sustainable silviculture and management guidelines. The third is the aim of the Stonepine Breeding Programme in Castile-Leon. The programme is the frame of various works started in the 90[th] as cooperation between the regional forest administration of Castile-Leon and the Forestry School (Polytechnic University of Madrid). The main topics of this programme were exposed by Catalán in the Nucis-Newsletter number 7 (1998); the current results and prospects are presented below.

The genetic improvement of stonepine points at two different purposes. Firstly, to meet the seed demand for afforestations with the selection and delimitation of seed stands to yield selected seeds, certified under EU and OECD regulation. Secondly, to search plus trees for vegetative propagation in specific plantations.

The necessity of seed stand delimitation is based on the traditional importance of stonepine, used in the afforestations performed during the 20[th] century, on degraded and eroded soils. The plantations of stonepine are justified by its low ecological requirements, easy nursery growing, high drought resistance and the commercial value of the expected cone yields. At present, since European reforestation programmes of farmland started in the 90[th], stonepine has become one of the most widely used species. It is the first one in the “Meseta Norte” region and fourth in the whole of Castile-Leon, with 11.836 new hectares planted between 1993 and 1997, a 20% increment of its former area. The aim is to achieve the best future forests in every site, including eroded or gypseous soils. This requires the use of certified seeds obtained in select stands with good phenotypes, adapted to each specific soil class and with a high cone production.

As basic short-term strategy, ten stands were identified and delimited since 1993 (Table 2) in order to obtain enough seed quantity, guarantying a broad genetic ran-

Table 2. Proposal of Selected Stonepine Seed Stands in Castile-Leon

Provenance Region	Code	Township (province)	Elevation (m)	Longitude	Latitude	Area (ha)
1 Meseta Norte	ES-23/01/001	Iscar (Valladolid)	750	4º31’W	41º21’N	360
	ES-23/01/002	Tordesillas (Valladolid)	680	4º57’W	41º30’N	106
	ES-23/01/004	La Parrilla (Valladolid)	855	4º34’W	41º33’N	20
	ES-23/01/005	Portillo (Valladolid)	850	4º32’W	41º28’N	59
	ES-23/01/006	Cogeces de Iscar (Valladolid)	800	4º57’W	41º25’N	22
	ES-23/01/007	Toro (Zamora)	680	5º27’W	41º31’N	45
	ES-23/01/008	Quintanilla de O. (Valladolid)	780	4º18’W	41º37’N	50
2 Tietar and Alberche Valleys	ES-23/02/001	El Hoyo de Pinares (Avila)	900-1000	4º21’W	40º31’N	26
	ES-23/02/002	El Hoyo de Pinares (Avila)	720-750	4º20’W	40º28’N	8
	ES-23/02/003	Cebreros (Avila)	600	4º22’W	40º25’N	75

ge in the seed sources, although without an experimental evaluation of its superiority.

The second line of the breeding programme started in 1989 with the fieldwork of plus tree selection, centred on high productive phenotypes, by annual measure of the individual trees' kernel yield in sampled stands. From the 135 sampled trees, grafted scions of 116 are included in clone banks. Of these, 35 are selected as plus trees by their cone yield superiority. At present, studies in the grafted clone banks are performed to characterize individual kernel yield, flowering and maturing phenology and cone and kernel biometry.

Comparative tests

In order to analyse the variability between different stonepine provenances or seed sources in their performance in afforestations, various field trials have been established in different Mediterranean countries. In 1995, the FAO Silva Mediterranea Network established an international field test with 35 provenances from seven Mediterranean countries, 17 of them from Spain. Four trial plots where planted in Spain. Castile-Leon took part with one of the plots in Tordesillas (Valladolid) and has seven seed sources, six of them proposed as selected seed stands. In the future, the information obtained in this trial is expected to allow the comparison of the genetic variability between provenances. Also it will allow to recommend the best seed sources for afforestations in each site (Martín & Prada, 1995).

Within the "Meseta Norte" provenance region, the evaluation of genetic variability in reproductive material of seed sources is performed in another trial. Seven experimental plots were established, which range all potential stonepine afforestation site types -from genuine sandy pìne-forest soil to clayey farmland and eroded marls-. In this test nine seed sources were represented, including the proposed selected seed stands. The objective of this comparative trial is to define tested seed stands in accordance with European (EU, OECD) and National legal standard. It should take into account the fitness (quantitative and adaptive traits, as survival, vigour, growth, adaptation to special soil conditions, biotic and abiotic risk resistance) and cone yield superiority.

Finally, the grafted plantations are used as test plots to estimate the genetic and environment control of flowering, fruitfulness and kernel yield. The objective was to test the pre-selected plus trees in the homogenous environment of cultivating and to select the best genotypes for controlled pollination.

View of the stonepine clonal bank "Meseta Norte"

Close up of a stonepine clone showing cones

Summary of performed labours and results

1. Seed stand selection

(a) Ecological and phenotype characterization of stonepine forests in Castile-Leon.

(b) Delimitation and characterization of representative stands of each defined type of stonepine forest.

(c) Based on this study, seven selected seed stands in the Meseta Norte region and three selected seed stands in the Tietar and Alberche Valleys region have been proposed to provide selected seed for afforestations. Currently, the catalogue of ten defined seed stands in Castile-Leon is in the approval phase in the Ministry of Environment.

Table 3. Cone yields in grafted stonepine plantations in Castile-Leon

Years after grafting	"El Carrascal" (278 trees/ha)		"Valdegalindo" (600 trees/ha)		
	kg/ha	Average (kg/tree)	kg/ha	Average (kg/tree)	Average of 20 plus tree
4	58	0.21			
5	183	0.66			
6	149	0.53			
7	157	0.57			
8	136	0.49			
9	460 *	1.65 *			
10	483 *	1.74 *	1,596	2,6	11,4
11	728 *	2.62 *	1,892	2,9	4,3
12			211	0,8	0,9

(*) Predicted values of flowering and maturing cones.
First 9 years in the older "Valdegalindo" plantation without yield information.

(d) Publication and popularisation of these topics to raise the conscience of potential users (foresters, nurserymen) about the advantage to use the best available seed sources.

2. Plus tree selection for vegetative reproductive material (graft scions)
(a) Preselection of good cone producing phenotypes (candidates) in natural stonepine stands in the Meseta Norte pine forests and evaluation by their kernel yield.

• 1989: selection of 100 candidate trees in 20 sample plots, with kernel yield measurement during a period of 8 years (1990-1997).

• 1991: installation of the first clonal bank "El Carrascal" with all 100 candidates. This grafted plantation is a clone archive to produce scions for future grafting.

• 1995-1996: selection of other 35 candidates in 7 new sample plots, with the objective to range the sample population over all stonepine growing sites in the region.

• In the results of the yield sampling, a dependence of the individual kernel production on the size and the growing space of the tree are observed, thus the selection criterion was the unshelled kernel yield referred to these two control parameters. Finally, 60 of the 135 pre-selected trees were selected to be propagated in the second clone bank. Actually, two twin plots were installed, one in Tordesillas, (Valladolid), the other in Puerta de Hierro, (Madrid).

(b) Studies in grafted plots.

• Individual yield measurement in "El Carrascal" clonal bank. On account of initial cultivation problems, a delay of growing and production was observed (Table 3).

• Growing and flowering phenology of stonepine in the "Meseta Norte" as main stonepine region in inland Spain has been studied in various plots.

• Controlled pollinations were started to obtain full-sib progenies for further trials, such as progeny tests. The disadvantage of stonepine is the three-year wait for mature seed.

Stonepine needle grafting

PROSPECTS

In the seed stand selection line, the results of the current comparative trial will offer the possibility to select the best for each afforestation site in the Douro Basin. Its seeds will be included in the official category of tested forest reproduction material.

In the clone selection line, the aim to test the grafted genotypes in homogenous conditions makes standarization of rootstocks, grafting and cultivation necessary. Currently, microcuttings are being developed from juvenile shoots to obtain clonal rootstocks which would allow "self-grafting" of the tested clones or the use of standard rootstocks in clone trials. The disadvantage of the cutting technique is the need of rejuvenation because of the rooting incapability of mature tissues in stonepine. Therefore it is not possible to obtain mature cuttings fit-to-test with early cone yields.

On the contrary, an accelerated maturation of propagules derived from cotyledons via tissue culture has been reported in plantlets of various pines and other species (M.S. Greenwood, personal communication). The organogenesis from cotyledons of half-sib or full-sib seedlings would allow premature progenies test and breeding value estimation of the tested progenitors. Furthermore, if the fructification of premature plants performs properly, it opens a way to obtain breeding generations in shorter period of time.

NOTES

[1] Values (without VAT) for nearly 16,000 ha of public stonepine forests in the province of Valladolid 1990-1998. The yearly cone yield rights of every pine stand is

sold at auction, based in appraisals of the cones still on the trees. The harvesting labours are performed by the purchaser, so the auction price is a direct forest owner's income.

[2] In the presence of nearby grown-up stonepines as pollinators.

REFERENCES

Abellanas, B., Butler, I., Monteagudo, F.J., 2000: Estudio de la rentabilidad económica de una parcela de injertos de pino piñonero a los nueve años de su instalación. Actas (II) del Primer Simposio del Pino Piñonero (*Pinus pinea* L.) febrero 2000, Valladolid: 101-110.

Catalán, G., 1998: Current Situation and Prospects of the Stonepines as Nut Producer. FAO - Nucis-Newsletter, number 7 December1998. IRTA, Reus: 28-32.

Gil, L., 1999: La transformación histórica del paisaje: la permanencia y la extinción local del pino piñonero. En: Marín, F.; Domingo, J. & Calzado, A. (editors): Los montes y su historia – una perspectiva política, económica y social. Universidad de Huelva: 151-185.

Gordo, J.; Mutke, S., Gil L., 2000: La mejora genética de *Pinus pinea L.* en Castilla y León.. Actas (II) del Primer Simposio del Pino Piñonero (*Pinus pinea* L.) febrero 2000, Valladolid: 21-32.

Martin S., Prada A., 1995. Ensayo de procedencias de pino piñonero (*Pinus pinea* L.) en España en: El pino piñonero como árbol productor de frutos secos en los países mediterráneos. I Reunión de la Red de Frutos Secos de la FAO sobre el Pino Piñonero. INIA- CIFOR. Madrid: 117-121.

Mutke, S.; Díaz-Balteiro, L., Gordo, J., 2000: Análisis comparativo de la rentabilidad comercial privada de plantaciones de *Pinus pinea* L. en tierras agrarias de la provincia de Valladolid. *Investigación Agraria- Sistemas y Recursos Forestales* Vol. 9(2) 2000 (in press).

Prada M.A., Gordo J., De Miguel J., Mutke S., Catalán G., Iglesias S., Gil L., 1997. Las regiones de procedencia de *Pinus pinea* L. en España. Organismo Autónomo de Parques Naturales. Madrid.

Sada, B.; Iglesias, S., Gil, L., 2000: Estudio de producción en un Banco Clonal de *Pinus pinea* L. de procedencia Cataluña Litoral para la selección de grandes productores de fruto. Actas (II) del Primer Simposio del Pino Piñonero (*Pinus pinea* L.) febrero 2000, Valladolid: 65-73.

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POLLEN TUBE GROWTH AND FERTILIZATION IN CAROB (*Ceratonia siliqua* L.)

INTRODUCTION

The present contribution is part of a four-year study that focused on flowering and fruiting of carob trees at different sites in Portugal (Haselberg 2000). Among the findings was the fact that only less than 60 % of the ovules in the pistils developed into seeds as a result of either failure of seed set or malformation respectively abortion of seeds during development.

High seed contents is essential for good crop quality in carob: seeds (kernels) constitute 10 % of the pod weight but make up more than 50 % of its value. Reasons for the sometimes high rate of seed failure are not yet exactly known, since the problem has not been paid adequate attention. Its economic relevance is emphasized by the fact that seed yield is positively correlated with the number of seeds per pod (Haselberg 2000). The objective of this study was to brighten the knowledge on the processes involved in pollen tube growth and fertilization and thus to contribute in finding strategies to improve the ovule-to-seed ratio.

MATERIALS AND METHODS

Experiments were carried out in South Portugal with trees of the female variety 'Mulata'. Flowering branches (3 to 5-years-old wood) were isolated with dense gaze cages. Inflorescences with flowers at anthesis were tagged and all but 5 to 7 synchronously developing flowers removed. Mature anthers were collected from various male trees and then stored in a warm environment until dehiscence. Hand pollination was carried out using a brush. Flowers were harvested between 6 hours and 11 days after hand pollination. For measurements of ovary and stigma longevity, flowers were hand pollinated 4, 6, 8, and 10 days after anthesis, maintained isolated and sampled after another 4 days. Sampled flowers were fixed immediately in ethanol, formalin, glacial acetic acid solution (9:0,5:0,5). For further preparation, the samples were rinsed with destillated water and then soaked in 8 N NaOH during 18 h for macerization. After complete removal of NaOH, flowers were fixed and stained in 0,1 N K_3PO_4 with alkaline aniline blue (0,1 %). Microscopic examinations included 11 to 15 flowers per treatment. Flowers were cut by hand into vertical and/or longitudinal transsections and surveyed for pollen tube growth; ovules and nucelli were measured and checked for pollen tubes. Anilin blue-induced fluorescence of callose sites was observed using a Zeiss standard microscope with Plan (2,5) and Neofluar (16, 25) objectives. An HG-lamp light source, filter combination 48-77-04 (providing blue-violet excitation), an excitation filter G 405 and colour partitioner 460 with longpass barrier 495 were applied.

RESULTS AND DISCUSSION

Pollen germination and tube growth characteristics

Non-germinated pollen were ellipsoid-formed, in contrast, germinated pollen grains were of approximately round shape and had a diameter of 31,3 µm (± 2,0). Findings are in accordance with former studies on carob pollen by several authors (Ferguson 1980, Ciampolini et al. 1988). In the hand-pollinated flowers an average number of 187 germinated pollen grains per stigma was assessed. Non-vital (unstained and tubeless) pollen grains were found on all stigmas. The pollen was not subjected to vitality tests, but a large variability in pollen germinability of 4 to 69 % has been reported for both wild and cultivated male genotypes (Sfakiotakis 1978, Ciampolini et al. 1988).

Pollen tubes penetrated between the papillae, passed the stigma tissue, and orientated towards the dorsal side of the bent pistil, where they formed dense tube bundles in the stylar transmitting tissue. Tubes that had entered the ovule cavity grew dorsally along the placenta surface and passed the ovules or entered them via micropyle in order to penetrate the nucellus. No specific tissue functioning as pollen tube pathway through the placenta was identified. Pollen tubes often displayed irregularities like fringes, knots, and callose plugs. In the ovule, penetrating tubes often formed knots in front of the nucellus (Fig. 1).

Fig. 1. Pollen tube growth in style and ovary: Pollen tube forming a knot in front of the penetrated nucellus. Bars represent 200 µm.